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Effects on Yolk Absorption.—As is apparent from an examination of the average yolk weights for the several groups, presented in table 1 and plotted in figure 1, there were no differences which were significant when tested by their probable errors, or which appeared consistently in the different series.

The high average value for the control chicks of shipment III, killed when 3 days old, is due to a single very large yolk. Omitting this one yolk would reduce the average yolk weight at 3 days of age for this lot from 3.44 ± 0.91 grams to 2.09 ± 0.39 grams, and for the total group of controls, from 2.60 ± 0.41 grams to 2.04 ± 0.19 grams.

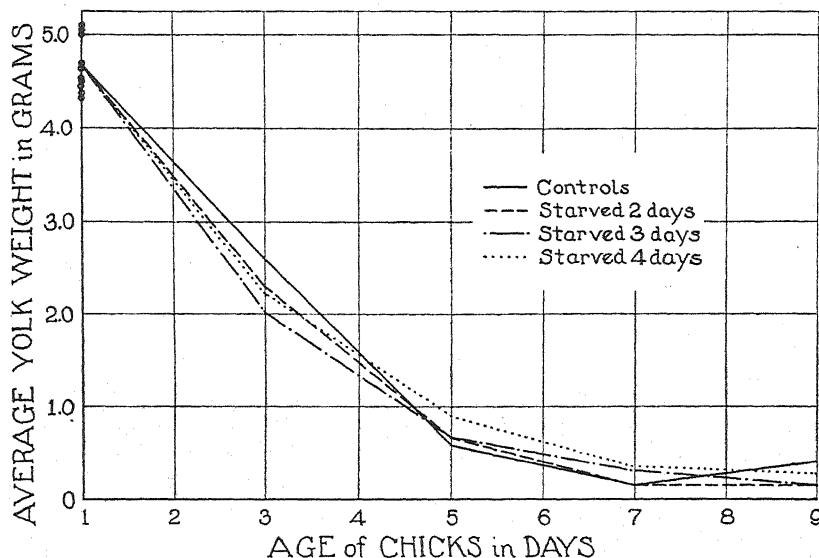


Fig. 1. The average yolk weights of chicks killed at different ages, from groups starved for various periods.

The differences between various samples of the same shipment, killed at 1 day of age, before the lots had received any differential treatment (and, therefore, not included in the table), are of about the same order of magnitude as the later differences. The average yolk weight at 1 day of age for various samples of 15 chicks each, all samples from the same shipment and having the same distribution of body weights, ranged from 4.32 ± 0.16 to 5.00 ± 0.34 grams. Since at 1 day of age the differences between the various averages are obviously due only to random sampling, the lines are all started from the grand average. The various averages are, however, shown as separate points on the graph (fig. 1). The fact that these differences are of about the same magnitude as any of the later ones confirms by actual sampling

the evidence from the computed probable errors, that starvation had no effect on yolk absorption marked or consistent enough to be significant with the numbers employed.

Effects on Mortality.—The mortality rates⁷ presented in table 2 bring out several points of interest. The first is that the chicks which were starved until 4 days old had a higher mortality than any of the other groups. This higher mortality is shown in both shipments II and III. The increase in mortality is due entirely to a higher death rate in the early weeks of life. After 3 weeks of age, there were no significant differences in mortality between the groups starved for varying periods and the control group.

Further, it is to be noted that in three of the lots, the chicks of shipment III suffered a higher mortality than those of shipment II, and that this difference also was evidenced in the first 3 weeks of life, there being no difference in the later mortality of the two shipments.

Finally, there appears to be no regularity in the differences between the male and female mortality in the various groups. Further discussion of the comparative mortality of the sexes will be deferred until the evidence from all the experimental groups can be considered together.

⁷ An explanation of the method of calculating these mortality rates should be given, since by killing samples for yolk determinations at different ages, the number of chicks was arbitrarily reduced on these dates. Therefore, for each lot, the number of chicks (multiplied by 100) dying within a period between the killing of samples was divided by the number of chicks at the beginning of the experiment, minus the number of chicks which had been killed up to that time. These mortality rates for the successive intervals of each particular lot were then combined by straight addition to obtain the mortality rate for the lot. This procedure amounts to assuming that the chicks which were killed would have suffered the same mortality rate as the rest of the chicks, and is as fair as any assumption which can be made. It should be stated that special precautions were taken that the samples killed should be unselected with respect to general vigor, or resistance to the particular handicap. This was ensured by making up the samples on paper, by day-old body weight and band number, without any reference to the appearance of the chicks.

In computing the probable errors of the mortality rates, the number of chicks after all the samples had been killed was used in every case as the value of n in the formula, $0.6745 \sqrt{\frac{pq}{n}}$. This means that the probable errors tabled are maximum values. Minimum values would be about seven-tenths of the tabled values since about half the chicks of each lot were killed for yolk determinations in the course of the experiment. It should also be noted that it was necessary to use the observed mortality rate in every case for the value of p in the formula, since there were no theoretical values, or values obtained from larger samples, which would be applicable to the various cases. Because of this difficulty, whenever the observed mortality rate happened to be zero, the probable error was automatically zero. Despite these limitations, it seemed worthwhile to include the probable errors, as approximations by which to judge the reliability of the differences observed.

Since all the chicks of shipment IV were killed for yolk weights during the first nine days of their life, mortality rates for the chicks of this shipment were not included. The data, however, are presented in table 21.

Effects on Body Weights of Survivors.—The average body weights at different ages, for the birds which survived the total experimental period of 20 weeks, are presented in table 3 and plotted in figures 2 and 3. The data for the two sexes are treated separately. As with the yolk weights, the plots show only the averages for the total groups given a particular treatment, while the table includes in addition the values for the separate lots.

TABLE 2
MORTALITY RATES* (PER 100) IN LOTS STARVED FOR VARIOUS PERIODS

Shipment No.	1 day (controls)	Length of starvation period from hatching		
		2 days	3 days	4 days
Total mortality (through 20 weeks)				
II.....	6.9±2.9	5.3±2.5	13.1±3.8	17.7±4.1
III.....	16.8±4.0	21.7±5.1	12.6±3.5	35.1±5.1
Total.....	12.0±2.5	11.5±2.6	12.8±2.6	26.4±3.3
Early mortality (hatching to 3 weeks of age)				
II.....	4.1±2.2	2.7±1.7	7.4±3.1	12.4±3.5.
III.....	14.2±3.7	14.6±4.4	12.5±3.5	30.0±4.9
Total.....	9.3±2.3	7.0±2.1	9.7±2.4	21.2±3.1
Later mortality (3 weeks through 20 weeks of age)				
II.....	2.8±1.9	2.6±1.7	5.7±2.7	5.3±2.5
III.....	2.6±1.8	7.1±3.1	0.0±0.0	5.1±2.3
Total.....	2.7±1.3	4.5±1.6	3.1±1.3	5.2±1.7
Comparative sex mortality (through 20 weeks)				
II♂'s.....	11.8±4.6	0.0±0.0	15.9±5.9	28.3±7.5
II♀'s.....	0.0±0.0	8.0±3.7	10.2±5.0	8.6±4.2
III♂'s.....	7.2±3.8	9.9±5.8	13.0±6.1	33.5±7.1
III♀'s.....	25.8±6.6	32.5±8.5	12.6±4.5	36.3±7.2
Total ♂'s.....	9.7±3.2	5.1±3.0	14.6±4.3	31.0±5.3
Total ♀'s.....	15.1±4.4	17.2±4.0	11.5±3.3	22.3±4.4

* The method of computing these rates is given in the footnote on page 00.

The graphs are plotted on semi-logarithmic paper, because of two advantages which this gives over plotting on arithmetic paper; the slopes of the lines indicate the *rate of gain* instead of the *absolute gain*, and the graphical differences between corresponding points on the several lines are more nearly in accordance with the probable errors of the differences at the various ages.

TABLE 3
AVERAGE BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM LOTS STARVED FOR VARIOUS PERIODS

Age of chicks	Shipment II				Shipment III				Total groups					
	Chicks starved for				Chicks starved for				Chicks starved for					
	1 day (con- trols)	2 days	3 days	4 days	1 day (con- trols)	2 days	3 days	4 days	Males	1 day (controls)	2 days	3 days	4 days	
1 day*	36.7	36.1	36.0	36.6	37.4	36.9	36.9	37.7*	54.0	54.6 ± 0.6	53.1 ± 0.8	51.2 ± 0.7	45.4 ± 0.6	
1 week	55.6	52.1	52.7	45.9	53.5	54.3	49.3	45.0	79.1	71.6 ± 1.4	77.1 ± 1.4	71.7 ± 1.4	71.7 ± 1.4	
2 weeks	84.0	80.3	81.0	74.3	75.6	77.7	72.2	69.3	80.6 ± 1.4	71.0 ± 2.5	113.0 ± 2.6	111.0 ± 2.6	111.0 ± 2.6	
3 weeks	123.4	122.4	117.5	116.7	118.9	110.3	107.9	106.0	121.4 ± 2.5	121.0 ± 2.5	161.0 ± 4.2	160.3 ± 3.9	160.3 ± 3.9	
4 weeks	160.7	172.0	165.8	165.5	173.4	162.6	166.4	155.6	169.8 ± 4.0	167.7 ± 4.3	223.5 ± 5.3	223.5 ± 5.3	223.5 ± 5.3	
5 weeks	235.2	239.8	231.7	227.6	241.6	225.4	215.3	217.7	238.2 ± 5.5	234.2 ± 5.5	323.4 ± 6.2	323.4 ± 6.2	323.4 ± 6.2	
6 weeks	312.2	311.8	314.9	314.9	292.3	281.3	281.3	281.3	313.4 ± 6.8	316.1 ± 7.4	380.0 ± 8.5	380.0 ± 8.5	380.0 ± 8.5	
6 weeks	432.0	429.2	415.0	401.8	397.0	354.1	357.5	376.7	416.2 ± 10.4	394.8 ± 10.0	388.4 ± 11.0	388.7 ± 10.3	388.7 ± 10.3	
8 weeks	628.5	622.0	604.5	592.7	575.5	527.9	596.7	622.0 ± 13.9	604.2 ± 14.0	580.2 ± 16.7	600.4 ± 13.9	580.2 ± 16.7	580.2 ± 16.7	
10 weeks	647.0	632.0	604.5	592.7	575.5	527.9	596.7	622.0 ± 13.9	604.2 ± 14.0	580.2 ± 16.7	600.4 ± 13.9	580.2 ± 16.7	580.2 ± 16.7	
12 weeks	932.0	880.0	850.0	876.0	827.2	827.5	850.0	850.0	888.9 ± 17.4	855.8 ± 18.7	894.4 ± 21.2	862.0 ± 16.5	862.0 ± 16.5	
16 weeks	1,419.0	1,330.0	1,338.7	1,311.8	1,285.3	1,250.0	1,182.5	1,182.5	1,357.6 ± 21.5	1,278.3	1,269.3 ± 21.5	1,269.3 ± 21.5	1,269.3 ± 21.5	
20 weeks	1,692.5	1,553.8	1,578.7	1,581.8	1,525.5	1,525.5	1,404.2	1,530.8	1,624.9 ± 22.3	1,540.8 ± 25.3	1,555.2 ± 18.0	1,555.2 ± 18.0	1,555.2 ± 18.0	
Probable error	±24.9	±39.8	±22.8	±28.0	±34.6	±28.1	±34.6	±28.1	±23.9	±23.9	±23.9	±23.9	±23.9	
Number of ♂s	20	13	15	11	17	11	12	12	37	24	27	27	23	
1 day	34.7	35.7	36.2	35.8	35.7	35.7	36.0	36.3	35.6 ± 0.5	35.7 ± 0.3	36.1 ± 0.3	36.0 ± 0.3	36.0 ± 0.3	
*	57.4	51.3	48.9	43.6	51.1	48.5	47.5	44.0	54.1 ± 0.9	50.5 ± 0.9	48.1 ± 0.5	43.8 ± 0.5	43.8 ± 0.5	
1 week	86.3	74.8	70.2	68.4	69.7	68.8	73.5	64.4	77.7 ± 1.7	73.0 ± 1.6	72.1 ± 1.1	66.9 ± 1.4	66.9 ± 1.4	
2 weeks	124.9	105.9	99.1	104.8	99.4	102.9	100.6	96.4	111.7 ± 3.1	105.0 ± 3.0	105.2 ± 2.1	101.7 ± 2.5	101.7 ± 2.5	
3 weeks	164.2	143.6	134.2	151.9	137.1	139.1	153.9	136.3	150.1 ± 4.4	142.2 ± 4.7	145.6 ± 3.7	145.6 ± 3.7	145.6 ± 3.7	
4 weeks	221.0	194.0	194.0	186.1	211.4	187.5	190.8	212.1	190.3	203.6 ± 6.4	193.1 ± 6.5	201.3 ± 4.8	203.6 ± 4.8	203.6 ± 4.8
5 weeks	288.4	245.3	245.3	287.2	250.1	246.6	273.5	255.5	208.6 ± 8.3	254.4 ± 7.9	260.9 ± 6.3	275.6 ± 6.1	275.6 ± 6.1	
6 weeks	371.2	358.0	337.7	390.0	320.7	307.5	344.5	352.6	345.0 ± 10.8	342.7 ± 10.0	373.3 ± 8.2	373.3 ± 8.2	373.3 ± 8.2	
8 weeks	555.4	545.2	50.47	569.7	494.3	478.0	534.3	620.9	523.7 ± 14.8	524.8 ± 13.5	521.9 ± 11.6	551.8 ± 9.5	551.8 ± 9.5	
10 weeks	770.0	733.9	721.3	770.5	683.6	668.0	755.2	711.8	725.2 ± 18.0	727.9 ± 15.6	729.4 ± 13.4	749.0 ± 11.2	749.0 ± 11.2	
12 weeks	1,036.2	1,034.8	996.0	1,023.7	943.6	927.0	989.0	977.3	988.1 ± 20.3	1,002.1 ± 16.6	901.9 ± 11.8	1,007.3 ± 11.8	1,007.3 ± 11.8	
16 weeks	1,269.7	1,269.6	1,246.7	1,263.7	1,185.7	1,100.0	1,281.1	1,125.5	1,218.2 ± 26.3	1,218.2 ± 21.9	1,230.0 ± 20.6	1,231.3 ± 14.5	1,231.3 ± 14.5	
20 weeks	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	1,367.7	
Probable error	±35.7	±22.1	±33.2	±15.7	±37.0	±41.3	±26.0	±24.6	±24.6	±24.6	±24.6	±24.6	±24.6	
Number of ♀s	13	23	15	19	14	10	21	11	27	33	36	36	30	

* Average weights of the starred lots just before they were given their first feed.

† Probable error of 20-week weight. It was not thought necessary to encumber the table with the probable error for the two shipments separately for the other ages.

It is apparent at once from an examination of the figures and tables, that in starved males and females alike there was a considerable loss of weight, roughly proportional to the length of the starvation period. The inequalities thus developed were maintained for the first few weeks, but were gradually effaced more or less completely.

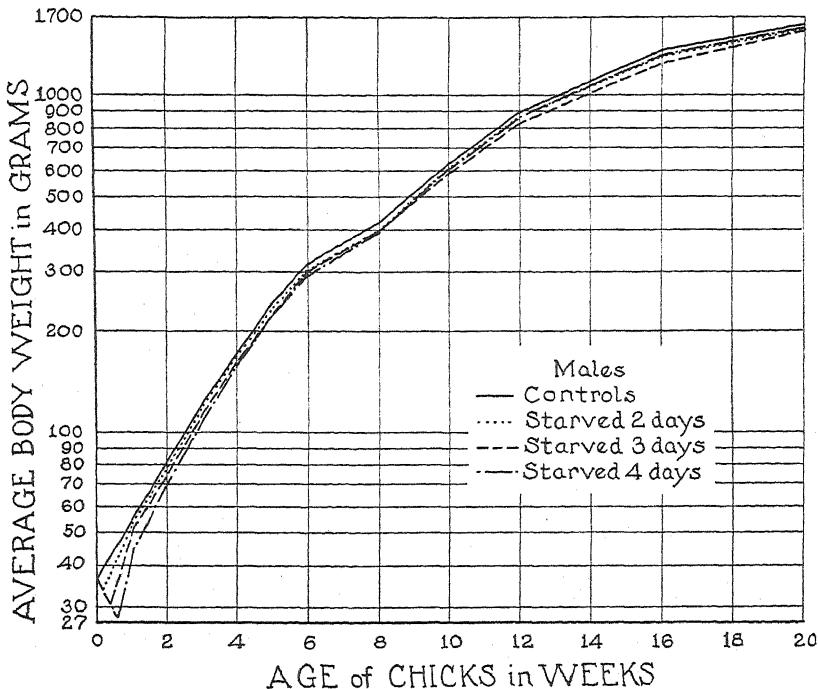


Fig. 2. The average body weights at different ages, of the males that survived the period of 20 weeks, from groups starved for various periods. All four curves start from the same point at one day old. The losses in weight suffered by the several groups during the starvation period were very closely proportional to the respective lengths of the periods. Therefore what appears as a single diagonal line at the left of the graph in reality represents the coincidence of the several curves during the starvation periods.

In the case of the females, at 20 weeks of age there were no significant differences in the average weights of the several groups. In the case of the males, all the groups which had their first feed delayed were at 20 weeks of age slightly, and about equally, below the group which was fed at 1 day of age. These differences are just on the border line of significance; the largest one, that between the chicks fed at 1 day of age and those fed at 3 days of age, is 97.1 ± 33.1 grams.

Examining these differences further, it is seen that while in shipment II all the starved lots had at 20 weeks of age smaller average

body weights than the control chicks, in shipment III the lots starved for 2 days and for 4 days had at about 10 weeks of age practically overcome the detrimental effect of the delay in first feeding. Therefore the evidence is not very strong for a permanent effect in the males, and there certainly was no permanent effect in the females.

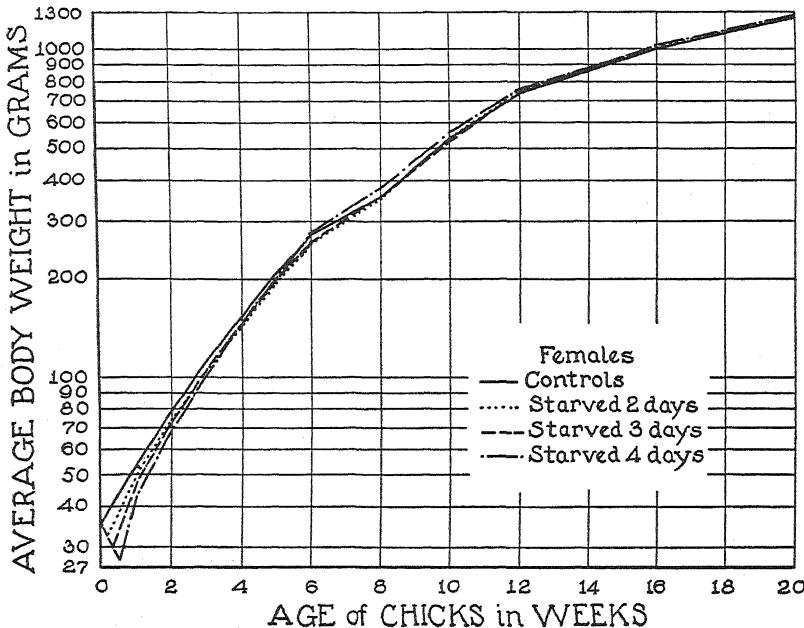


Fig. 3. The average body weights at different ages, of the females that survived the period of 20 weeks, from groups starved for various periods. All four curves start from the same point at one day old. The losses in weight suffered by the several groups during the starvation periods were very closely proportional to the respective lengths of the periods. Therefore what appears as a single diagonal line at the left of the graph in reality represents the coincidence of the several curves during the starvation periods.

It is possible that the tendency toward a more permanent effect in the males which, to anticipate somewhat, was also evidenced in the case of several other handicaps, may have been related to the more severe competition among the cockerels.

The standard deviations and coefficients of variation are given in tables 4 and 5 and are of the same order of magnitude as those found by other investigators working with chicks; see Pearl (1917) and Latimer (1924). The relative variability is of the same order of magnitude as that found in rats; see Jackson (1913) and King (1918,

1919). In all the series the relative variability is lowest, about 10 per cent, at the beginning of independent life, rises to a maximum of around 25 to 30 per cent, and then decreases again.

TABLE 4
STANDARD DEVIATIONS OF BODY WEIGHTS OF DIFFERENT AGES, OF THE CHICKS
THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM GROUPS
STARVED FOR VARIOUS PERIODS

Age of chicks	Length of starvation period from hatching			
	1 day (controls)	2 days	3 days	4 days
Males				
1 day.....	2.8± 0.2	3.0± 0.3	3.0± 0.3	3.1± 0.3
1 week.....	5.7± 0.4	5.6± 0.5	5.5± 0.5	4.2± 0.4
2 weeks.....	12.4± 1.0	10.4± 1.0	10.6± 1.0	10.1± 1.0
3 weeks.....	22.8± 1.8	18.9± 1.8	20.2± 1.9	18.5± 1.8
4 weeks.....	35.7± 2.8	31.3± 3.0	32.5± 3.0	27.4± 2.7
5 weeks.....	50.1± 3.9	39.7± 3.9	47.5± 4.4	37.6± 3.7
6 weeks.....	61.5± 4.8	53.7± 5.2	65.1± 6.0	50.9± 5.1
8 weeks.....	93.5± 7.3	70.0± 7.7	64.8± 7.8	73.2± 7.3
10 weeks.....	125.6± 9.8	102.0± 9.9	120.7± 11.1	98.5± 9.8
12 weeks.....	157.3± 12.3	136.0± 13.2	163.5± 15.0	117.4± 11.7
16 weeks.....	194.3± 15.2	158.5± 15.4	198.0± 16.2	128.3± 12.0
20 weeks.....	201.5± 15.8	183.7± 17.9	186.3± 17.1	132.6± 13.2
Number of ♂s.....	37	24	27	23
Females				
1 day.....	3.5± 0.3	3.0± 0.3	3.1± 0.3	2.8± 0.3
1 week.....	6.9± 0.6	7.1± 0.6	4.7± 0.4	5.0± 0.4
2 weeks.....	13.0± 1.2	13.9± 1.2	9.9± 0.8	11.7± 1.0
3 weeks.....	24.2± 2.2	25.9± 2.2	18.4± 1.5	20.0± 1.7
4 weeks.....	34.3± 3.1	40.4± 3.4	28.5± 2.3	29.7± 2.6
5 weeks.....	49.4± 4.5	55.0± 4.6	42.4± 3.4	39.3± 3.4
6 weeks.....	63.6± 5.8	67.4± 5.6	56.2± 4.5	49.5± 4.3
8 weeks.....	83.0± 7.6	90.5± 7.5	76.8± 6.1	66.3± 5.8
10 weeks.....	113.7± 10.4	114.7± 9.5	103.5± 8.2	78.5± 6.7
12 weeks.....	138.4± 12.7	132.9± 11.0	118.9± 9.5	90.9± 7.9
16 weeks.....	156.1± 14.3	141.1± 11.7	139.8± 11.1	96.1± 8.4
20 weeks.....	202.6± 18.6	186.9± 15.5	183.4± 14.6	117.6± 10.2
Number of ♀s.....	27	33	36	30

The chicks which had been starved for the first 4 days after hatching were (in the females after about 6 weeks, and in the males after about 10 weeks) somewhat less variable than the controls, both in respect to absolute and relative variability. The results further show that the relative variability of the females at 20 weeks of age was slightly in excess of that of the males in every group. The absolute variability was substantially the same for the two sexes.

TABLE 5

COEFFICIENTS OF VARIATION OF BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS
THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM GROUPS
STARVED FOR VARIOUS PERIODS

Age of chicks	Length of starvation period from hatching			
	1 day (controls)	2 days	3 days	4 days
Males				
1 day.....	7.6±0.6	8.2±0.8	8.2±0.8	8.4±0.8
1 week.....	10.4±0.8	10.5±1.0	10.7±1.0	9.3±0.9
2 weeks.....	15.4±1.2	13.1±1.3	13.7±1.3	14.1±1.4
3 weeks.....	18.8±1.5	15.6±1.6	17.8±1.7	16.7±1.7
4 weeks.....	21.0±1.7	18.7±1.9	20.1±1.9	17.1±1.7
5 weeks.....	21.0±1.7	17.0±1.7	21.2±2.0	16.9±1.7
6 weeks.....	19.6±1.6	17.7±1.8	29.0±2.9	17.3±1.8
8 weeks.....	22.5±1.9	20.0±2.0	21.8±2.1	18.8±1.8
10 weeks.....	20.2±1.6	16.9±1.7	20.8±2.0	16.4±1.7
12 weeks.....	17.7±1.4	15.9±1.6	19.8±1.9	13.6±1.4
16 weeks.....	14.3±1.1	12.3±1.2	15.6±1.5	9.3±0.9
20 weeks.....	12.4±0.9	11.9±1.2	12.2±1.1	8.5±0.8
Number of ♂s.....	37	24	27	23
Females				
1 day.....	9.8±0.9	8.4±0.7	8.6±0.7	7.8±0.7
1 week.....	12.8±1.2	14.1±1.2	9.8±0.8	11.4±1.0
2 weeks.....	16.7±1.6	19.0±1.6	13.7±1.1	17.5±1.6
3 weeks.....	21.7±2.1	24.7±2.2	17.5±1.4	19.7±1.8
4 weeks.....	22.8±2.2	28.4±2.5	19.6±1.6	20.4±1.8
5 weeks.....	24.3±2.3	28.5±2.5	21.1±1.8	19.3±1.7
6 weeks.....	23.7±2.3	26.5±2.3	21.5±1.8	18.0±1.6
8 weeks.....	24.1±2.3	26.4±2.3	22.2±1.9	17.8±1.6
10 weeks.....	21.7±2.1	21.9±1.9	19.8±1.6	14.2±1.3
12 weeks.....	19.1±1.8	18.3±1.6	16.3±1.3	12.1±1.1
16 weeks.....	15.8±1.5	14.1±1.2	14.1±1.1	9.5±0.8
20 weeks.....	16.5±1.6	15.3±1.3	14.9±1.2	9.6±0.8
Number of ♀s.....	27	33	36	30

EXPERIMENTS ON ADMINISTRATION OF POISONS

For the particular purposes of these experiments the exact dosage or mode of action of the poisons used was not a primary consideration. The only requirement was the determination of a constant dose which, when administered to all the individuals of a group, would be lethal to a considerable proportion of the individuals, so that comparisons could be made between individuals which survived and those which succumbed, and between survivors and controls.

Various considerations guided the choice of the particular poisons. Nicotine sulfate was used because of the widespread interest in its supposed stunting effect and because of its frequent use as a vermicide for poultry. Mercuric chloride and arsenic trioxide were tested to determine whether heavy metallic salts, whose elimination is slow, might bring about more permanent effects than the other handicaps used. Sodium chloride was of particular interest because of its universal presence in the diet, and its poisonous action in excessive amounts.

Experimental Procedure.—By preliminary trials, dosages were determined which were lethal to about 30 per cent of the individuals of a group. The most satisfactory way to administer all of the poisons was found to be by mouth, in small gelatine capsules (No. 5). The capsules were dipped in olive oil, and placed well down the throat.

The dosage adopted for nicotine sulfate was a capsule full (0.2 cc.) of a 3 per cent solution. In the preliminary trials an 8 per cent solution had killed every chick to which it was given, a 6 per cent solution had killed 70 per cent of the chicks, and a 4 per cent solution had killed 50 per cent of the chicks. With the 3 per cent solution there was an almost immediate reaction, practically identical for every chick, a complete coma lasting for about fifteen minutes, but very few deaths.

With the other poisons no immediate reaction could be seen, but in a large proportion of the cases of early deaths, certain characteristic postmortem appearances were found associated with each specific type of poisoning.⁸

In preliminary trials, a capsule full of dry sodium chloride killed every chick to which it was given; a capsule half full killed about 50 per cent of the chicks; while of the chicks receiving a capsule one-quarter full none died. The dosage adopted therefore was a capsule one-third full, or 0.06 gram of the pure dry salt. This dose effected a mortality of 18 per cent in the chicks of shipment II; in the chicks of shipment III, however, the mortality was 70 per cent. Since the

⁸ The majority of the dead chicks from the nicotine group had very blue shanks and beak, the kidneys congested, and in several cases the right side of the heart filled with black clotted blood; those from the group given sodium chloride had the rectum and cloaca enormously distended, and very pale kidneys; in the chicks which died from the group given mercuric chloride the ureters appeared choked with urates; the chicks which died from the 2 per cent dose of arsenic trioxide showed dark lesions on the liver, but with the smaller doses of arsenic there were no noticeable abnormalities.

latter group received the poison before receiving any feed,⁹ two lots in shipment IV were given this same dose. Both lots were given the salt at 24 hours of age, but one lot was fed before it was poisoned, to determine whether the higher mortality obtained in shipment III could be attributed to the difference in age of chicks at the time of receiving poison, or to its administration when the alimentary tract was empty. In the lot that had not been fed before poisoning eighteen chicks died, while in the lot that was fed before poisoning only 11 chicks died. This difference is not nearly so great as that between the mortality suffered by the corresponding lots of shipments II and III.

With both metallic poisons the preliminary trials had indicated a capsule full (0.2 cc.) of a 2 per cent solution as a satisfactory dose. When this dose was used, however, on the chicks of shipment II, so high a mortality resulted that the doses were reduced for the corresponding lots of the later shipments. The chicks of shipments III and IV were given a 1 per cent solution of mercuric chloride, and had a mortality rate practically identical with that of the lot getting the 2 per cent dose, between 35 and 40 per cent. The decrease in dose was evidently counterbalanced by a slight inferiority of the later chicks, or by the later chicks not having been fed before receiving poisons, or by the combined effect of both differences.

All of the chicks given the 2 per cent solution of arsenic died, so that the chicks which were to have been killed at 3 days old were used instead for a second arsenic lot,¹⁰ which was given a 0.5 per cent solution of arsenic. Since the resulting mortality was about 50 per cent, for the corresponding lots of shipments III and IV the dose was reduced to a 0.25 per cent solution. In shipment III, 14 per cent of the chicks died, while in shipment IV there were no deaths. Thus slight differences in the size of dose apparently had a greater influence upon mortality in the case of arsenic trioxide than in the case of mercuric chloride.

Effects on Yolk Absorption.—From the averages presented in table 6 and plotted in figure 4, it would appear at first glance that marked differences were obtained in the yolk weights of the various samples

⁹ Since the preliminary trials had shown no difference in results whether the poisons were given at one or two days of age, the chicks of shipment II were not given their poisons until the second day. It was then found to be of advantage, in order to save rehandling of the chicks on the second day, to give the poisons on the first day, before the chicks were put under the hovers. This procedure was accordingly followed with shipments III and IV.

¹⁰ This explains the fact that no samples were killed from shipment II for yolk determinations at 3 days of age.

killed at 3 days old, all of the poisoned groups except that given sodium chloride having yolks averaging less than the control group. However, the differences are not significant, the largest ones being less than twice their probable error. The appearance arises largely from the influence of the one very large yolk already noted in the control group. As stated above, omitting this one observation would make the average for the controls of shipment III 2.09 ± 0.39 grams, and for the total group of controls 2.04 ± 0.19 grams. In the samples killed at 5 and at 7 days old, all the poisoned groups had yolks averaging slightly larger than the controls, but these differences also are not significant. At 9 days old, there was no regularity in the comparisons, nor were any of the differences significant.

TABLE 6

AVERAGE YOLK WEIGHTS (IN GRAMS) OF SAMPLES KILLED AT DIFFERENT AGES,
FROM LOTS GIVEN DIFFERENT POISONS

Shipment No.	Controls	Mercuric chloride	Sodium chloride (not fed first)	Sodium chloride (fed first)	Arsenic trioxide	Nicotine sulfate
Chicks killed at 3 days old						
III.....	* 3.44 ± 0.91	1.71 ± 0.15	2.04 ± 0.27	1.68 ± 0.25
IV.....	2.01 ± 0.18	1.66 ± 0.16	2.54 ± 0.23	1.58 ± 0.23	2.05 ± 0.17	1.99 ± 0.25
Total.....	2.60 ± 0.41	1.68 ± 0.11	2.54 ± 0.23	1.58 ± 0.23	2.04 ± 0.15	1.86 ± 0.18
Chicks killed at 5 days old						
II.....	0.31 ± 0.06	0.68 ± 0.20	0.98 ± 0.28	0.19 ± 0.06	0.94 ± 0.18
III.....	0.47 ± 0.17	0.58 ± 0.15	1.36 ± 0.47	0.68 ± 0.20
IV.....	0.82 ± 0.16	0.77 ± 0.20	1.16 ± 0.22	1.29 ± 0.31	0.74 ± 0.19	0.99 ± 0.25
Total.....	0.58 ± 0.09	0.68 ± 0.11	1.16 ± 0.22	1.17 ± 0.22	0.79 ± 0.17	0.88 ± 0.14
Chicks killed at 7 days old						
II.....	0.12 ± 0.07	0.45 ± 0.24	0.16 ± 0.19	0.22 ± 0.08	0.11 ± 0.07
III.....	0.25 ± 0.14	0.94 ± 0.42	1.45 ± 0.34	0.28 ± 0.11	0.80 ± 0.19
IV.....	0.09 ± 0.04	0.12 ± 0.19	0.40 ± 0.09	0.36 ± 0.10	0.05 ± 0.06	0.09 ± 0.14
Total.....	0.14 ± 0.04	0.48 ± 0.16	0.79 ± 0.18	0.27 ± 0.07	0.16 ± 0.04	0.29 ± 0.08
Chicks killed at 9 days old						
II.....	0.00 ± 0.00	0.29 ± 0.17	0.17 ± 0.07	0.54 ± 0.31
III.....	* 1.31 ± 0.77	0.35 ± 0.19	0.63 ± 0.33	0.17 ± 0.06
IV.....	0.22 ± 0.04	0.08 ± 0.04	0.60 ± 0.19	0.09 ± 0.04	0.43 ± 0.18	0.00 ± 0.00
Total.....	0.41 ± 0.20	0.20 ± 0.07	0.60 ± 0.19	0.18 ± 0.04	0.49 ± 0.16	0.22 ± 0.10

* Note, from the raw data in table 20, that each of these two high values is due to a single extreme case.

From a careful study of the various comparisons to be made, of the various poisoned groups with controls and with each other, and of the several lots which were subjected to the same handicap, it appears that there is only one difference occurring with sufficient regularity to warrant emphasis. The average yolk weights of 10 of the 12 different samples of chicks given sodium chloride were larger than the corresponding averages for the controls. It is unfortunate that from shipment III a complete series of samples was not obtained. As

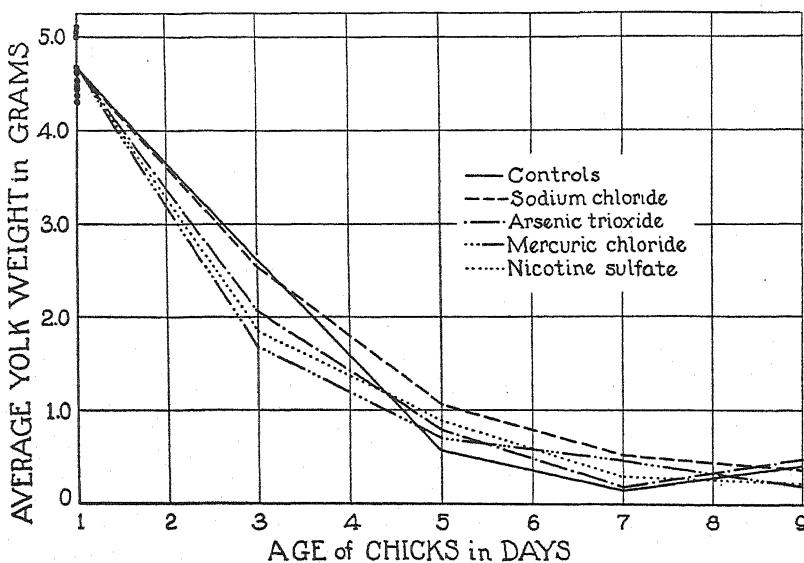


Fig. 4. The average yolk weights of chicks killed at different ages, from groups given various poisons.

has been noted above, the early mortality of this lot was very high, so that it seemed important to keep as many survivors as possible, rather than to kill samples for yolk weights. Two complete series of samples were obtained from shipment IV.

In all shipments, chicks which were given sodium chloride *before* receiving their first feed averaged heavier yolks at every age than the controls. The chicks which were poisoned *after* they were fed also had heavier yolks than the control lot at 5 and at 7 days of age. At 3 and at 9 days of age, however, the yolks averaged slightly less than the controls. These two samples furnished the only exceptions to a generalization that yolk absorption was slower in chicks which received sodium chloride.

Effects on Mortality.—Some references have already been made to the mortality figures in connection with the discussion of the dosages employed. The rates are presented in table 7. The main additional points to be mentioned are the same generalizations which were found to hold for the lots deprived of food for different periods; namely, that there were no consistent differences in the male and female mortality rates, and that the subjection of the chicks to the early handicaps did not cause an increased mortality rate after three weeks of age. The total groups given mercuric chloride and nicotine sulfate had later mortality rates slightly higher than the controls, while those given sodium chloride and arsenic trioxide had mortality rates slightly below the controls. These comparisons, however, did not hold for all of the separate shipments, and were not significant in any case.

TABLE 7

MORTALITY RATES (PER 100) OF THE POISONED AND OPERATED LOTS

Shipment No.	Controls	Mercuric chloride	Sodium chloride	Arsenic trioxide	Nicotine sulfate	Yolk removed
Total mortality (through 20 weeks)						
II.....	6.9±2.9	38.5±5.2	18.4±4.3	50.3±5.7	30.5±5.3	25.0±5.1
III.....	16.8±4.0	36.6±5.3	70.0±4.1	13.6±4.2	23.8±4.8	58.1±6.1
Total.....	12.0±2.5	38.8±3.7	44.8±3.4	30.3±3.8	26.9±3.6	41.3±4.3
Early mortality (hatching to 3 weeks of age)						
II.....	4.1±2.2	36.0±5.1	15.7±4.0	50.3±5.7	19.1±4.5	18.7±4.7
III.....	14.2±3.7	25.8±4.9	70.0±4.1	13.6±4.2	18.4±4.3	45.2±6.1
Total.....	9.3±2.3	32.3±3.5	43.7±3.4	30.3±3.8	18.6±3.1	31.8±4.0
Later mortality (3 weeks through 20 weeks of age)						
II.....	2.8±1.9	2.5±1.6	2.7±1.6	0.0±0.0	11.4±3.7	6.3±2.9
III.....	2.6±1.8	10.8±3.4	0.0±0.0	0.0±0.0	5.4±2.6	12.9±4.1
Total.....	2.7±1.3	6.5±1.8	1.1±0.9	0.0±0.0	8.3±2.2	9.5±2.6
Comparative sex mortality (through 20 weeks)						
II♂'s.....	11.8±4.6	36.0±7.2	18.2±5.5	41.0±8.8	31.2±7.4	21.1±6.2
II♀'s.....	0.0±0.0	41.0±7.4	18.9±6.9	59.1±8.0	30.9±7.5	30.8±8.7
III♂'s.....	7.2±3.9	41.1±7.4	75.0±6.3	10.4±6.0	21.3±7.2	82.5±6.6
III♀'s.....	25.8±6.6	32.4±8.0	66.7±5.4	16.3±5.5	26.9±6.3	42.9±9.0
Total ♂'s.....	9.7±3.2	42.2±5.3	41.4±5.0	26.9±5.5	26.4±5.2	44.5±5.6
Total ♀'s.....	15.1±4.4	35.8±5.4	48.4±4.8	33.0±5.0	28.6±4.7	37.0±6.3

Effects on Body Weights of Survivors.—Turning now to the body weights of the survivors, which are presented in table 8 and plotted in figures 5 to 8, several points are to be observed. The first is that the only group which throughout the entire period remained below the control group in all four sub-groups (both sexes separately, and both lots separately) was the group given mercuric chloride. At

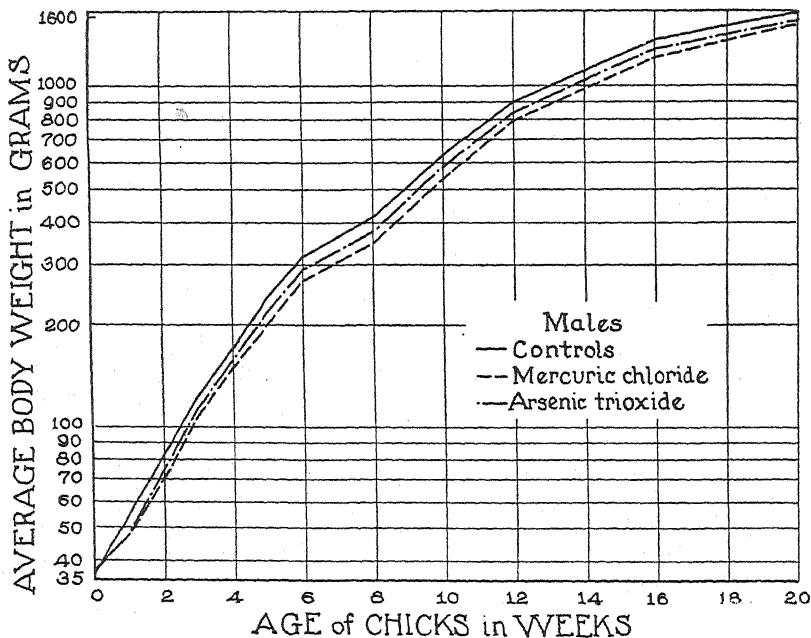


Fig. 5. The average body weights at different ages, of the males that survived the period of 20 weeks, from groups given heavy metallic poisons.

20 weeks of age the males of shipment II which had been given mercuric chloride averaged 152 ± 38 grams lower in body weight than the controls. The difference between the average weights of the females from these same lots was only 53 ± 50 grams. For the corresponding lots in shipment III the difference was 62 ± 72 grams in the case of the males, and 162 ± 59 grams in the case of the females. Combining the lots, the differences for both sexes are just on the border line of significance, being 112 ± 41 grams for the males and 112 ± 42 grams for the females. Thus, while the differences are not significant, the fact that they are all in the same direction is suggestive, and indicates that this point may be worthy of further investigation.

The only other poison which gave uniform results in all sub-groups was sodium chloride. The survivors from this poison showed no material differences in body weight as compared with the controls, in either sex in either of the lots. This was true in spite of the fact that the mortality was very markedly higher in the lot from shipment III than in the lot from shipment II.

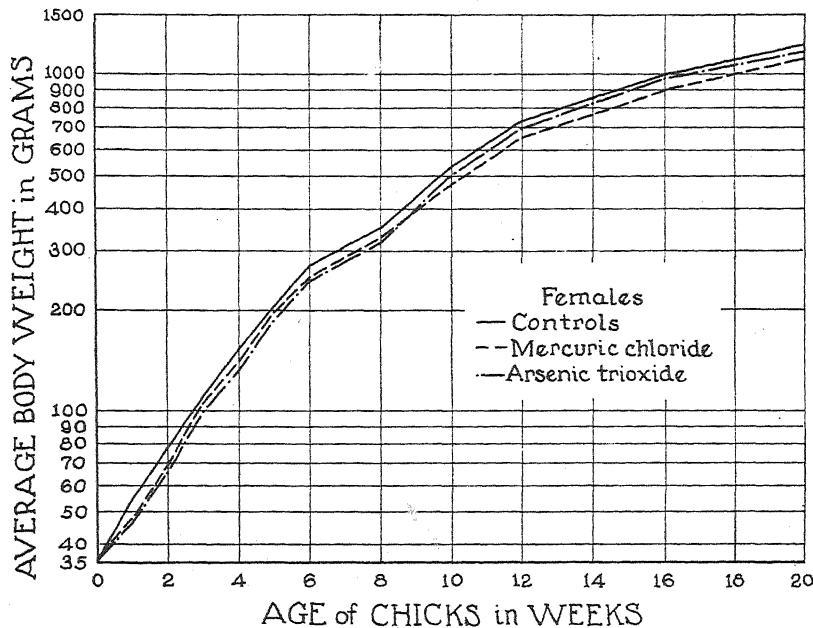


Fig. 6. The average body weights at different ages, of the females that survived the period of 20 weeks, from groups given heavy metallic poisons.

The two arsenic lots gave interesting results, which may be correlated with the differences in mortality effected by the slightly different dosages employed. The males of shipment II (which received the 0.5 per cent dose of arsenic and suffered a 50 per cent mortality) remained markedly lower in weight than the control lot throughout the period of 20 weeks (actual difference, 208 ± 66 grams at 20 weeks), while the males of shipment III (which received the 0.25 per cent dose of arsenic and had only 13 per cent mortality) equalled the controls in body weight.

The females of shipment II given arsenic trioxide equalled the controls after about 10 weeks of age, and those of shipment III after about 2 weeks. Among the females, therefore, while there was eventual recovery in both shipments, the smaller dose apparently allowed

TABLE II
AVERAGE BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS SURVIVED THE PERIOD OF 20 WEEKS, FROM POISONED AND OPERATED

Age of chicks	Shipment III												Total groups													
	Shipment II				Shipment III				Shipment IV				Controls			Mercuric chloride			Sodi n chloride		Arsenic trioxide		Nicotine sulfate		Yolk removed	
	Controls	Mercuric chloride	Sodium chlorite	Arsenic trioxide	Controls	Mercuric chloride	Sodium chlorite	Yolk removed	Controls	Mercuric chloride	Sodium chlorite	Yolk removed	Controls	Mercuric chloride	Sodium chlorite	Yolk removed	Controls	Mercuric chloride	Sodium chlorite	Yolk removed	Controls	Mercuric chloride	Sodium chlorite	Yolk removed		
1 day.....	36.7	37.4	36.5	36.0	35.5	36.6	37.0	36.8	37	38.4	36.7±0.3	37.2±0.4	38.5±0.4	37.1±0.4	38.0±0.4	36.2±0.4	36.0±0.4	36.2±0.4	36.0±0.4	36.2±0.4	36.0±0.4	36.2±0.4	36.2±0.4			
1 week.....	55.6	48.9	51.5	41.9	63.5	45.9	44.8	50	44.0	54.6±0.6	47.5±1.1	51.9±1.2	47.5±1.1	50.0±0.8	42.4±0.8	50.0±0.8	42.4±0.8	50.0±0.8	42.4±0.8	50.0±0.8	42.4±0.8	50.0±0.8				
2 weeks.....	84.9	74.9	81.4	68.0	72.3	67.6	66.5	75	59.6	80.6±1.4	71.2±2.1	72.5±2.1	72.1±1.7	61.7±1.4	61.7±1.4	61.7±1.4	61.7±1.4	61.7±1.4	61.7±1.4	61.7±1.4	61.7±1.4	61.7±1.4				
3 weeks.....	123.4	115.2	106.6	108.3	98.5	118.9	104.8	120	82.6	121.4±2.5	108.3±3.1	120.5±3.6	110.5±3.6	108.4±2.9	108.4±2.9	108.4±2.9	108.4±2.9	108.4±2.9	108.4±2.9	108.4±2.9	108.4±2.9	108.4±2.9				
4 weeks.....	166.7	166.5	170.5	147.4	145.0	146.1	173.4	152.0	168	117.6	109.8±4.0	147.5±4.4	167.0±6.1	159.9±6.8	150.9±6.3	159.0±7.3	159.0±7.3	159.0±7.3	159.0±7.3	159.0±7.3	159.0±7.3	159.0±7.3	159.0±7.3			
5 weeks.....	235.2	209.6	203.3	183.6	236.9	202.9	241.6	239	184.6	216.2	200.5±5.5	200.5±6.3	233.1±8.2	218.9±9.6	205.4±6.5	193.3±5.4	193.3±5.4	193.3±5.4	193.3±5.4	193.3±5.4	193.3±5.4	193.3±5.4	193.3±5.4			
6 weeks.....	312.2	277.4	316.5	266.7	279.2	314.9	247.3	297.3	301	313.4±6.5	297.8±9.0	312.5±11.0	287.2±12.9	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0	263.5±11.0			
8 weeks.....	432.0	357.3	434.7	355.7	324.5	375.7	397.6	337.0	375.0	382	378.0±10.4	347.6±12.5	423.3±14.9	372.0±16.5	352.1±11.6	351.3±12.2	351.3±12.2	351.3±12.2	351.3±12.2	351.3±12.2	351.3±12.2	351.3±12.2	351.3±12.2			
10 weeks.....	647.0	535.5	644.4	538.6	501.8	558.0	592.7	528.0	677.5	608	426.0	622.0±13.9	631.9±17.6	573.4±26.1	543.8±18.2	525.0±16.7	525.0±16.7	525.0±16.7	525.0±16.7	525.0±16.7	525.0±16.7	525.0±16.7	525.0±16.7			
12 weeks.....	932.0	790.9	880.0	790.0	745.5	815.3	838.2	774.0	817.6	867	614.0	888.9±17.9	782.9±22.2	908.3±34.8	783.3±23.0	765.0±23.3	765.0±23.3	765.0±23.3	765.0±23.3	765.0±23.3	765.0±23.3	765.0±23.3	765.0±23.3			
16 weeks.....	1,419.0	1,247.3	1,381.2	1,230.0	1,175.5	1,288.0	1,285.5	1,180.0	1,232.5	1,310	1,024.0	1,357.6±21.5	1,215.2±26.0	1,362.5±24.0	1,277.1±22.7	1,184.8±27.1	1,126.0±24.0	1,126.0±24.0	1,126.0±24.0	1,126.0±24.0	1,126.0±24.0	1,126.0±24.0	1,126.0±24.0	1,126.0±24.0		
20 weeks.....	1,692.6	1,540.0	1,642.4	1,484.3	1,484.3	1,484.3	1,484.3	1,484.3	1,484.3	1,484.3	1,484.3±6.0	1,572.0±22.3	1,512.9±34.8	1,023.8±27.8	1,535.9±48.8	1,474.8±30.7	1,488.0±30.2	1,488.0±30.2	1,488.0±30.2	1,488.0±30.2	1,488.0±30.2	1,488.0±30.2	1,488.0±30.2			
P. E. *	±24.0*	±28.7	±31.0	±61.5	±23.0	±34.6	±23.0	±34.6	±23.0	±54.6	±69	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2	±76.2			
Number of ♂'s.....	20	11	17	7	11	15	17	10	4	10	.5	37	.5	21	.5	17	.5	21	.5	21	.5	20	.5			
F	34.7	34.3	35.9	35.2	36.0	34.8	36.5	38.3	38.2	35.0	35.6±0.5	35.0±0.5	36.4±0.5	37.1±0.5	36.5±0.5	36.8±0.5	36.8±0.5	36.8±0.5	36.8±0.5	36.8±0.5	36.8±0.5	36.8±0.5	36.8±0.5			
1 day.....	57.4	45.9	54.6	40.6	50.3	43.8	51.1	48.4	46.7	47.3	54.0±0.9	47.2±1.5	50.7±1.0	45.9±1.1	50.4±0.9	43.1±0.8	43.1±0.8	43.1±0.8	43.1±0.8	43.1±0.8	43.1±0.8	43.1±0.8	43.1±0.8			
1 week.....	86.3	79.4	80.6	62.2	77.4	64.4	69.7	68.6	67	60.4	77.7±1.7	69.5±2.6	73.7±1.8	65.9±1.6	73.2±1.7	62.5±1.6	62.5±1.6	62.5±1.6	62.5±1.6	62.5±1.6	62.5±1.6	62.5±1.6	62.5±1.6			
3 weeks.....	124.9	102.4	119.9	92.8	118.9	98.6	90.4	105.9	104.0	100	91.6	111.1±3.1	104.3±4.5	112.0±2.8	99.0±2.6	112.3±3.0	95.3±3.0	95.3±3.0	95.3±3.0	95.3±3.0	95.3±3.0	95.3±3.0	95.3±3.0	95.3±3.0		
4 weeks.....	164.2	135.6	160.8	131.6	133.6	157.7	141.3	137.1	143.3	148.0	129	134.6	150.2±4.4	139.6±6.4	154.4±4.4	130.2±3.6	154.0±4.8	138.2±5.4	154.0±4.8	154.0±4.8	154.0±4.8	154.0±4.8	154.0±4.8	154.0±4.8	154.0±4.8	
5 weeks.....	221.0	186.0	216.6	191.6	208.2	194.7	187.5	200.9	200.9	180	187.0	203.0±6.4	194.1±9.6	208.4±6.6	183.4±5.0	205.4±6.6	191.1±8.1	191.1±8.1	191.1±8.1	191.1±8.1	191.1±8.1	191.1±8.1	191.1±8.1	191.1±8.1		
6 weeks.....	288.4	241.0	279.8	247.8	267.8	284.9	250.1	257.8	265.5	239	251.4	268.0±6.3	249.6±11.3	272.5±9.0	241.5±6.7	265.4±7.7	268.5±11.7	268.5±11.7	268.5±11.7	268.5±11.7	268.5±11.7	268.5±11.7	268.5±11.7	268.5±11.7		
8 weeks.....	371.2	327.8	344.0	322.7	344.0	35.2	36.0	34.8	36.5	38.3	38.2	37	35.0	35.6±10.8	323.4±16.3	335.5±12.3	315.2±6.6	340.5±14.1	340.5±14.1	340.5±14.1	340.5±14.1	340.5±14.1	340.5±14.1	340.5±14.1	340.5±14.1	
10 weeks.....	555.4	501.1	563.6	548.0	531.0	523.3	494.3	486.0	505.5	48	510.6	523.0±14.8	466.8±22.1	534.6±17.0	497.6±12.9	530.4±13.0	517.4±14.5	517.4±14.5	517.4±14.5	517.4±14.5	517.4±14.5	517.4±14.5	517.4±14.5	517.4±14.5		
12 weeks.....	770.0	703.3	788.2	722.0	753.0	721.1	683.6	693.6	695.5	670	697.5	725.2±16.0	646.8±27.7	741.8±21.2	690.0±15.3	736.8±15.6	710.0±23.5	710.0±23.5	710.0±23.5	710.0±23.5	710.0±23.5	710.0±23.5	710.0±23.5	710.0±23.5		
16 weeks.....	1,086.2	965.3	1,000.0	1,052.7	891.0	945.6	851.0	943.6	966.3	988.5	986.3	988.5±20.5	899.5±33.3	962.4±28.8	984.4±20.5	985.9±27.4	985.9±27.4	985.9±27.4	985.9±27.4	985.9±27.4	985.9±27.4	985.9±27.4	985.9±27.4			
20 weeks.....	1,297.7	1,214.4	1,321.8	1,220.0	1,234.0	1,214.0	1,204.4	1,185.7	1,023.0	1,199.1	1,158.1±20.5	1,225.5±26.3	1,113.7±33.3	1,245.5±27.9	1,172.9±22.9	1,173.4±26.9	1,173.4±26.9	1,173.4±26.9	1,173.4±26.9	1,173.4±26.9	1,173.4±26.9	1,173.4±26.9	1,173.4±26.9			
P. E. *	±35.7*	±34.8	±39.8	±25.2	±34.0	±33.7	±37.0	±47.5	±42.5	±28	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7	±47.7			
Number of ♀'s.....	13	9	11	5	10	9	14	10	11	16	8	27	19	22	21	25	21	25	21	25	21	25	21	25		

* Probable errors of the 20-week weight.

Effects on Body Weights of Survivors.—Turning now to the body weights of the survivors, which are presented in table 8 and plotted in figures 5 to 8, several points are to be observed. The first is that the only group which throughout the entire period remained below the control group in all four sub-groups (both sexes separately, and both lots separately) was the group given mercuric chloride. At

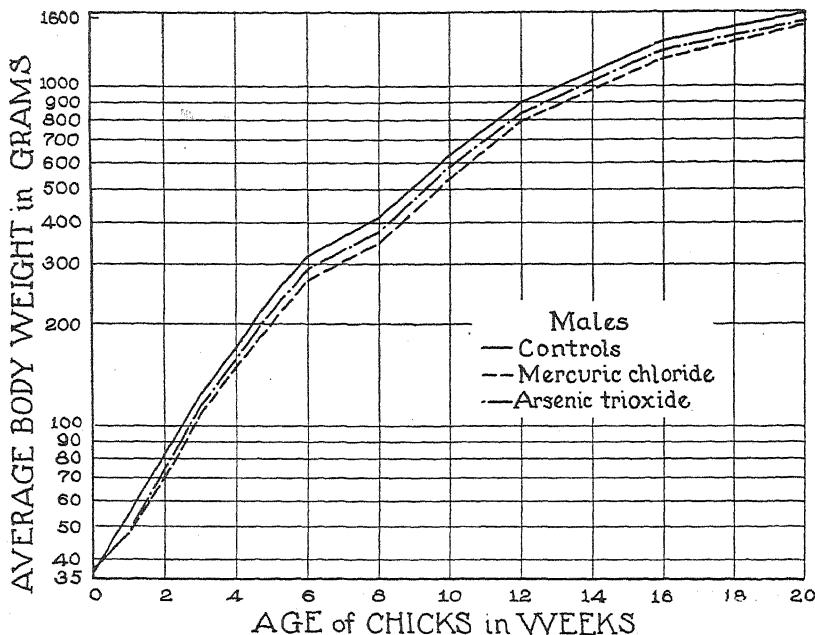


Fig. 5. The average body weights at different ages, of the males that survived the period of 20 weeks, from groups given heavy metallic poisons.

20 weeks of age the males of shipment II which had been given mercuric chloride averaged 152 ± 38 grams lower in body weight than the controls. The difference between the average weights of the females from these same lots was only 53 ± 50 grams. For the corresponding lots in shipment III the difference was 62 ± 72 grams in the case of the males, and 162 ± 59 grams in the case of the females. Combining the lots, the differences for both sexes are just on the border line of significance, being 112 ± 41 grams for the males and 112 ± 42 grams for the females. Thus, while the differences are not significant, the fact that they are all in the same direction is suggestive, and indicates that this point may be worthy of further investigation.

The only other poison which gave uniform results in all sub-groups was sodium chloride. The survivors from this poison showed no material differences in body weight as compared with the controls, in either sex in either of the lots. This was true in spite of the fact that the mortality was very markedly higher in the lot from shipment III than in the lot from shipment II.

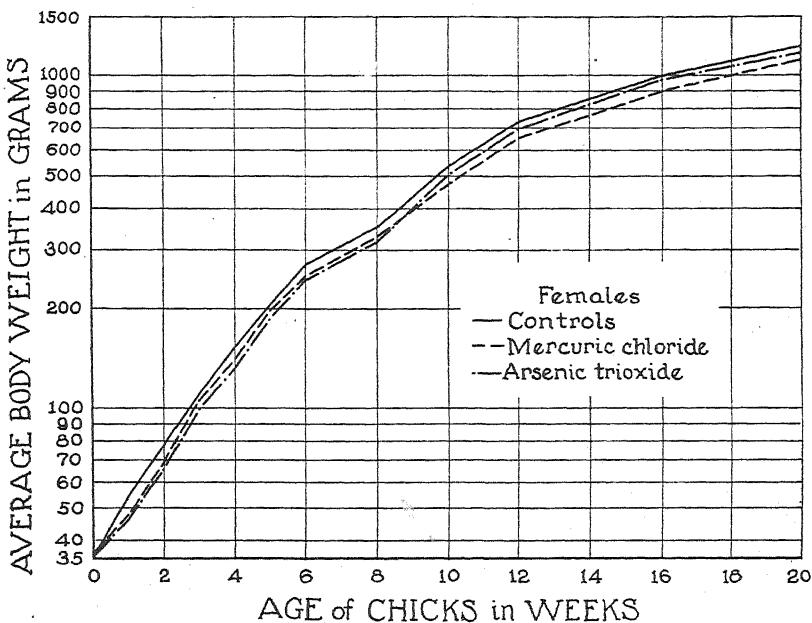


Fig. 6. The average body weights at different ages, of the females that survived the period of 20 weeks, from groups given heavy metallic poisons.

The two arsenic lots gave interesting results, which may be correlated with the differences in mortality effected by the slightly different dosages employed. The males of shipment II (which received the 0.5 per cent dose of arsenic and suffered a 50 per cent mortality) remained markedly lower in weight than the control lot throughout the period of 20 weeks (actual difference, 208 ± 66 grams at 20 weeks), while the males of shipment III (which received the 0.25 per cent dose of arsenic and had only 13 per cent mortality) equalled the controls in body weight.

The females of shipment II given arsenic trioxide equalled the controls after about 10 weeks of age, and those of shipment III after about 2 weeks. Among the females, therefore, while there was eventual recovery in both shipments, the smaller dose apparently allowed

a more rapid recovery. When the two shipments are combined they give a spurious appearance of showing a less complete recovery, as compared with the controls, because of the fact that there happened to be three times as many females in the survivors of this lot in shipment III as in shipment II (and shipment III was inferior to shipment II in all lots, as evidenced by both mortality and body weights), so that the average of the total group is heavily weighted with the inferior birds. When the data are properly analyzed, therefore, the females show the more complete recovery than the males which was noted in the cases of starvation.

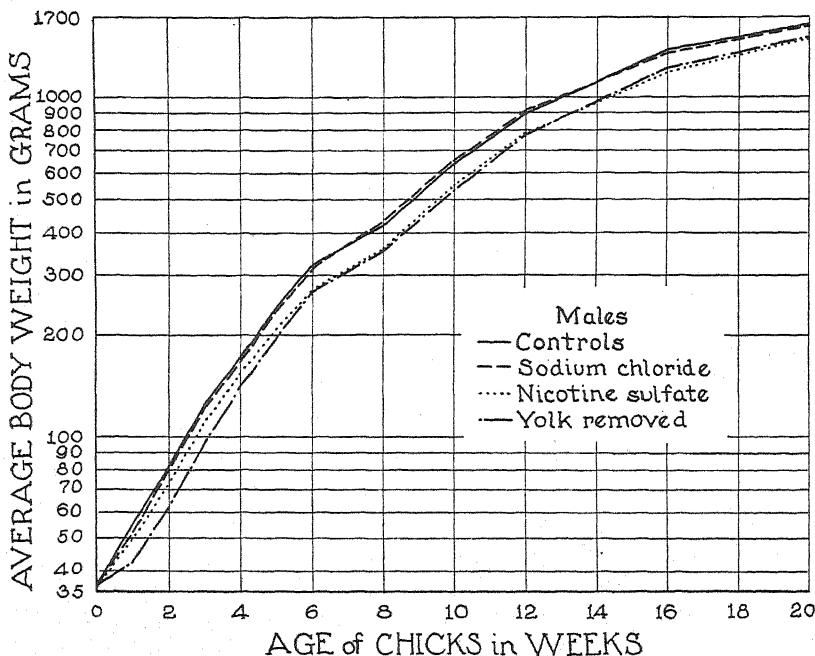


Fig. 7. The average body weights at different ages, of the males that survived the period of 20 weeks, from the operated group, and from groups poisoned by substances normally or frequently administered to chicks.

This same result was obtained with nicotine sulfate. The males remained below the controls in both lots; the differences were 228 ± 54 grams and 59 ± 78 grams for the separate shipments, and 150 ± 38 grams for the total group. The females again showed complete recovery in both shipments.

The main points to be noted from the standard deviations and coefficients of variation in tables 9 and 10 are that there were no significant differences in the standard deviations of the various groups,

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EFFECTS OF EARLY HANDICAPS ON CHICKENS AS MEASURED BY YOLK ABSORPTION AND BODY WEIGHT TO TWENTY WEEKS OF AGE^{1,2}

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GENERAL PROBLEM AND LITERATURE

That growth is markedly influenced by environmental factors is well known. The precise effects and optimum conditions have been the subject of so many investigations that reference can be made only to those which have the most direct bearing on the experiments reported here. Most of the previous investigations have been concerned with the results obtained when the experimental subjects were kept continuously under the particular environmental conditions. The point with which the present experiments are primarily concerned is the question of how permanent the effects of environmental handicaps are, when those handicaps are maintained for only a short time, during the early life of the subjects.

There are in the literature numerous indications that individuals may recover completely, even when adverse conditions have been

¹ Contribution No. 9 from the Division of Poultry Husbandry, University of California Agricultural Experiment Station.

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long continued. Thus Osborn and Mendel (1915) found that rats whose growth had been suppressed for long periods by inanition still retained the capacity to grow, so that growth recurred at periods far beyond the age at which it ordinarily ceases.

Several investigators had previously found that growth could be retarded or even suppressed for brief periods at an early age without permanent effects on weight. Minot (1891) states that a young guinea pig may lose one-third of its weight from intestinal catarrh and make good the loss later, and cites Pagliani (1879) as showing that undersized children brought up in poverty may recover in the most surprising manner if placed under favorable circumstances. Hatai (1907, p. 320) found that "so far as the weight of the body and central nervous system are concerned, the effect of a twenty-one day period of partial starvation on albino rats thirty days old is eventually completely compensated." Boas (1912) noted that in children, while retardation of early growth is made up by abnormally rapid development at a later period, an unduly prolonged retardation produced permanent effects. Aron (1910, 1914) working with dogs and rats, and Morgulis (1911, 1913) with salamanders, observed that periods of inanition were followed by periods of very vigorous growth.

Several investigations have been concerned with the effects of starvation on particular organs (Aron, 1911; Jackson, 1913, and 1917; Stewart, 1916), measuring the differential loss of weight suffered by the several organs and tissues during starvation.

As Aron (1911) points out, most of the scanty experimental evidence heretofore collected on this topic pertains to subjects which have been stunted in their growth by underfeeding, and he raises the question as to whether the conclusions drawn from such a mode of inhibition apply equally well to other conditions of retarded growth.

Shapiro (1905) chloroformed young kittens twice a day and observed a marked retardation in growth, and later recovery after the treatments were stopped. However, he used only three kittens, alternating them as experimental and control animals. A few observations were made by Richon and Perrin (1908) on the retardation of growth in rabbits by injections of nicotine and the recovery after the injections were discontinued. In this case too, only a few animals were used and individuals are cited, some of which compare very favorably with the controls, while others do not.

All of these experiments seem to have been conducted with the point of view typified by the following quotation from Osborne and Mendel (1915, p. 453): "It should be noted that the resumption of

growth has not been as perfect in every instance as in the typical records here presented. A positive result in these cases is far more valuable than a failure, because the latter may arise from a variety of extraneous, as well as inherent, causes which he [the investigator] cannot control or discover. In prolonged stunting, the animals may sometimes reach a precarious condition in which their vitality may become impaired beyond the possibility of recovery. They are sensitive to noxious influences and cannot be expected to show great resistance under the conditions of limited diet. Subsequent statistics may show damage hitherto unappreciated. The factor of safety must be small."

STATEMENT OF SPECIFIC PROBLEM

A consideration of these facts made it desirable to study the effects of a variety of handicaps, and to measure average results upon groups of individuals compared with control groups. Further, it seemed important to determine the relationship between the permanence of the effects and the extent of the injury as measured by the mortality rate, in groups where the handicap was made severe enough to produce a considerable mortality.

The subjects chosen were baby chicks. This choice made possible the use of two measures of the effects of the experimental treatment—growth rates, and the course of yolk absorption.

The handicaps to which the various lots were subjected included overheating; chilling; complete starvation for different lengths of time after hatching; total deprivation of water for a given period; certain poisons, the particular ones chosen being nicotine sulfate, mercuric chloride, arsenic trioxide, and sodium chloride; and a major operation, in this case the removal of the unabsorbed yolk at one day of age.

PLAN OF EXPERIMENTS

The chicks were Single Comb White Leghorns obtained from a large commercial hatchery, since it was important to have a large representative population which had hatched during a short interval of time. Eggs which are all set at the same time hatch throughout an interval of some 36 hours, and the incubators usually are not opened until the hatch is practically complete. By special arrangement for these experiments, the incubators were opened and chicks furnished which hatched within a 6-hour period.

For the major portion of the experiments approximately 1,800 chicks were used. The exigencies of the experimental treatments to be described made it physically impossible to handle them all in one day. Therefore they were obtained in three separate shipments of 600 chicks each, on October 4, 6, and 8, 1927. These will hereafter be referred to as shipments I, II, and III.

The chicks of shipment I were divided into four equivalent lots. In making up the lots all the chicks were weighed individually when the average age was 24 hours. As they were weighed they were sorted according to weight to the nearest gram. After the entire shipment had been weighed, the chicks in each weight class were distributed equally among the four lots. This procedure was adopted to ensure having the lots as comparable as it was possible to make them at the time the differential experimental treatments were given.

The same procedure was followed for shipments II and III, except that each shipment was divided into nine lots. Eight different experimental treatments were given to eight of the lots, respectively, from shipment II. The same eight treatments were given to eight lots, respectively, from shipment III. One lot from each shipment was used as a control.

The reasons for these differences in procedure between shipment I and shipments II and III arose from the fact that the treatments given the several lots in shipment I (overheating, chilling, and deprivation of water) required that each lot be housed separately for the first few days. This was not essential for the experimental treatments given shipments II and III (starvation,⁴ administration of poisons, and removal of yolk at 1 day of age). Hence, advantage was taken of the opportunity to duplicate each experimental treatment on two smaller lots rather than simply carry it out on one larger one. Where more than one lot was given a particular experimental treatment, these may hereafter on occasion be referred to together as an experimental group.

All chicks were individually marked with serially numbered wing bands, and were housed in 8 x 8 foot colony houses having concrete outdoor runs. Each house was equipped with a Lyons thermostatically controlled electric hover. One hundred and fifty chicks were put under each hover, which was of the size to accommodate 350. All the lots of a given shipment (except in shipment I, and those after the

⁴ All starved lots were held in chick boxes until the time of their first feeding. This is a standard practice among poultrymen in delayed feeding. For these experiments it seemed desirable, since it prevented the results from being complicated by factors other than inanition, such as litter eating, toe picking, etc.

first few days) were distributed equally among the different houses. This ensured having all environmental conditions except the differential experimental treatments identical, and also put handicapped and control chicks in competition with each other. From the standpoint of the ultimate bearing of these experiments on the general problem of the permanence of effects of early handicaps, this procedure seems preferable to the isolation of differentially treated groups.

At 8 weeks of age the chicks were all moved to larger open front houses without hovers. The sexes were separated, and all of a given sex from a given shipment were put together in one house. About this time all lots suffered mildly from coccidiosis. The outbreak was not serious but, as an inspection of the graphs presented later will show, it caused a slight and approximately equal dip in all the growth curves.

All chicks were fed the same standard ration. A dry mash consisting of ground grains, fish meal, and mineral supplements was before the chicks constantly. Scratch grains were fed in addition after the chicks were one week old. Fresh greens were supplied daily.

Unless otherwise stated all lots were given feed and water when twenty-four hours old. Starvation refers to the withholding of both food and water.

Individual body weights were secured at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 20 weeks of age. The weights were taken by means of a special Toledo scale, giving readings by 1-gram divisions for objects under 500 grams weighed in a small scoop at the end of an extended beam, and by 10-gram divisions for heavier objects weighed on the main platform.

It was not practicable to make observations on the actual progress of yolk absorption in living chicks, in the large numbers which preliminary work had shown to be necessary because of the variability of the process. Therefore an indication of the course of yolk absorption was obtained by killing samples of chicks from each group at different ages and weighing the unabsorbed yolks. The yolk sacs with their contents were carefully dissected out and weighed to the nearest hundredth of a gram on a chemical balance. Yolks weighing less than 0.01 gram were classified as absorbed.

The samples which were killed for yolk weights were always chosen so that the frequency distributions of day-old body weights were the same for all the different groups, and also the same as that of the total group at the start of the experiment. This is an important preau-

tion because of the correlation between day-old body weights and yolk weights, the evidence of which will be presented later.

It was planned to kill 15 chicks from each experimental group at each of the following ages: 1, 3, 5, 7, and 9 days. Because in particular instances this procedure could not be carried out completely, an additional shipment of chicks was obtained, which will be referred to as shipment IV. These chicks were divided into 15 lots of 40 chicks each and were used for yolk determinations of the entire series of handicaps previously tested, and for three additional ones.

All chicks that died were examined for unabsorbed yolk, and for any abnormal postmortem appearances.

All the raw data of the experiments, including yolk weights, mortality data, and body weights of survivors, are presented in tables 20 to 23,⁵ in the appendix.

The most convenient plan for analyzing these data is to discuss first certain more or less natural groupings of the various handicaps. After these are discussed separately, the data of the various experiments are combined to allow more general comparisons to be made, of handicapped with control chicks, and of survivors with chicks which died.

EXPERIMENTS ON STARVATION FOR DIFFERENT LENGTHS OF TIME AFTER HATCHING

Since the newly hatched chick has so large a store of nutriment within its body in the form of yolk, the belief has arisen that not only does the chick not require other food during the first two or three days after hatching, but that other food actually interferes with the normal absorption of the yolk, and is thereby injurious to the chick. Practically all recommendations to poultrymen (e. g., Alder, 1924; Vandervort, 1925; Clickner, 1927) contain emphatic warnings that if chicks are fed too early, or too much, the yolk will not be absorbed, and digestive troubles and subsequent losses will result. The period recommended for withholding all feed varies from 48 to 72 hours after hatching, with strictly limited feed for two to three weeks thereafter.

The only figures on yolk absorption we have been able to find in the literature are those given by Virchow (1891) and Schilling and

⁵ These tables were for the purposes of publication reduced photographically beyond the point of ready visibility. In this way it was possible to make available for any other workers all the actual raw data. If desired, the figures may of course be re-enlarged photographically, or they may be easily made out with a reading lens.

Bleecker (1928). Virchow (p. 288) gives the unabsorbed yolk weights for six chicks only, as follows:

12 hours old.....	5.34	grams
36 " "	3.24	"
3 days old.....	2.50	"
3-4 " "	0.60	"
5-6 " "	0.05	"
6-7 " "	0.43	"

Schilling and Bleeker's paper appeared after the experiments here reported had been completed. Their investigation was directed to the solution of the same general problem of the influence of food consumption upon the rapidity of yolk absorption. They used 75 chicks. All were starved for the first three days. One group was thereafter given food *ad libitum*. The other group was given approximately one-fourth as much feed. The conclusion reached was that through the period of their observations, which included the first nine days after hatching, there was no evidence that the quantity of food consumed influenced the rate of yolk absorption. As will be seen, their conclusion, based upon the quantity of feed given, is in complete agreement with the results obtained in the experiments reported here, on the basis of differing periods of starvation.

Experimental Procedure.—In preliminary trials two periods of starvation—24 hours and 72 hours—had been tested. No significant differences appeared in the rate of yolk absorption in the two groups; there were, however, some indications of a difference in growth rate. It therefore seemed desirable to test a more complete series of periods of starvation, up to the point where some deaths from starvation occurred. This point was found to be 5 days after hatching.⁶

The starved chicks were weighed again just before they were given their first feed, to determine the loss of weight which occurred during the starvation period.

⁶ It is interesting to note that autopsies of the starved chicks which died revealed in many cases considerable quantities of unabsorbed yolk, suggesting that the yolk of itself is not sufficient to support life. All of the starved chicks that died showed a characteristic appearance of the alimentary tract, as compared with autopsy findings in dead chicks from other lots at comparable ages. The same appearance was found in chicks from the starved lots killed for yolk determinations before their first feeding. In starved chicks the gizzard was always small and soft walled, and the intestines black and shriveled.

Further evidence that the presence of normal unabsorbed yolk does not indicate that other food is unnecessary was found in the fact that chicks from which the yolk had been removed at 1 day of age withstood starvation nearly as long as unoperated birds. This point is being investigated further, and will be reported more completely at a later date.

TABLE 1

AVERAGE YOLK WEIGHTS (IN GRAMS) OF SAMPLES KILLED AT DIFFERENT AGES,
FROM LOTS STARVED FOR VARIOUS PERIODS

Shipment No.	Length of starvation period from hatching					
	1 day (controls)	2 days	3 days	4 days	5 days	4 days, given water
Chicks killed at 3 days old						
III.....	*3.44±0.91	2.35±0.46	2.54±0.55	2.02±0.34
IV.....	2.01±0.18	2.22±0.44	1.67±0.18	2.30±0.29	2.06±0.26	2.01±0.22
Total.....	2.60±0.41	2.27±0.32	2.00±0.25	2.20±0.22	2.06±0.26	2.01±0.22
Chicks killed at 5 days old						
II.....	0.31±0.04	0.40±0.05	0.48±0.08	1.26±0.34
III.....	0.47±0.17	0.95±0.34	0.65±0.18	1.02±0.17
IV.....	0.82±0.16	0.58±0.12	0.77±0.12	0.59±0.10	0.84±0.11	0.97±0.24
Total.....	0.58±0.09	0.66±0.13	0.66±0.08	0.90±0.12	0.84±0.11	0.97±0.24
Chicks killed at 7 days old						
II.....	0.12±0.05	0.12±0.07	0.57±0.13	0.47±0.16
III.....	0.25±0.14	0.22±0.08	0.09±0.04	0.49±0.14
IV.....	0.09±0.04	0.12±0.04	0.24±0.09	0.17±0.05	0.17±0.06	0.20±0.07
Total.....	0.14±0.04	0.15±0.03	0.30±0.07	0.36±0.07	0.17±0.06	0.20±0.07
Chicks killed at 9 days old						
II.....	0.00±0.00	0.06±0.04	0.40±0.25	0.53±0.33
III.....	*1.31±0.54	0.13±0.08	0.01±0.01	0.07±0.02
IV.....	0.22±0.06	0.22±0.07	0.03±0.01	0.06±0.02	0.00±0.00	0.46±0.25
Total.....	0.41±0.20	0.15±0.04	0.16±0.09	0.27±0.15	0.00±0.00	0.46±0.25

*Note from the raw data in table 20 that each of these two high values is due to a single extreme case.

or of the two sexes. The high value of the standard deviation of the males of the arsenic trioxide group (97 ± 38 grams higher than the controls) is due to a single very small bird which weighed only 710 grams at 20 weeks of age. Omitting this individual would reduce the standard deviation to a value only 20 ± 31 grams above that for the controls, or 222 ± 26 grams. In every case except this one, the relative variability was slightly greater for the females than for the males, as was found to be true in the starved groups.

TABLE 9

STANDARD DEVIATIONS OF BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS
THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM POISONED
AND OPERATED GROUPS

Age of chicks	Controls	Mercuric chloride	Sodium chloride	Arsenic trioxide	Nicotine sulfate	Yolk removed
Males						
1 day.....	2.8 ± 0.2	2.9 ± 0.3	2.8 ± 0.3	2.5 ± 0.3	3.0 ± 0.3	2.5 ± 0.3
1 week.....	5.7 ± 0.4	7.8 ± 0.8	8.0 ± 0.8	6.7 ± 0.8	5.5 ± 0.6	5.4 ± 0.6
2 weeks.....	12.4 ± 1.0	14.2 ± 1.5	14.9 ± 1.6	13.0 ± 1.5	11.4 ± 1.2	8.9 ± 1.0
3 weeks.....	22.8 ± 1.8	21.0 ± 2.2	24.3 ± 2.5	25.2 ± 2.9	19.3 ± 2.0	15.9 ± 1.7
4 weeks.....	35.7 ± 2.8	29.7 ± 3.1	41.3 ± 4.3	41.3 ± 4.8	29.5 ± 3.1	24.6 ± 2.6
5 weeks.....	50.1 ± 3.9	42.6 ± 4.4	55.6 ± 5.8	58.9 ± 6.8	44.4 ± 4.6	36.0 ± 3.8
6 weeks.....	61.5 ± 4.8	61.0 ± 6.4	74.5 ± 7.7	78.7 ± 9.1	70.5 ± 7.3	52.3 ± 5.6
8 weeks.....	93.5 ± 7.3	84.9 ± 8.8	101.1 ± 10.5	100.6 ± 11.6	79.0 ± 8.2	81.1 ± 8.6
10 weeks.....	125.6 ± 9.8	119.3 ± 12.4	110.7 ± 11.5	159.8 ± 18.5	123.9 ± 12.9	110.4 ± 11.8
12 weeks.....	157.3 ± 12.3	150.6 ± 15.7	142.6 ± 14.8	212.7 ± 24.6	149.7 ± 15.6	154.2 ± 16.4
16 weeks.....	194.3 ± 15.2	176.7 ± 18.4	163.3 ± 17.0	260.7 ± 30.2	184.4 ± 19.2	199.5 ± 21.3
20 weeks.....	201.5 ± 15.8	236.3 ± 24.6	188.9 ± 19.7	298.2 ± 34.5	208.7 ± 21.7	200.3 ± 21.4
Number of ♂'s.....	37	21	21	17	21	20
Females						
1 day.....	3.5 ± 0.3	3.4 ± 0.4	3.3 ± 0.3	3.1 ± 0.3	3.4 ± 0.3	2.8 ± 0.3
1 week.....	6.9 ± 0.6	9.9 ± 1.1	7.1 ± 0.7	7.3 ± 0.8	6.5 ± 0.6	5.0 ± 0.6
2 weeks.....	13.0 ± 1.2	16.7 ± 1.8	12.4 ± 1.2	11.0 ± 1.1	12.3 ± 1.2	10.0 ± 1.2
3 weeks.....	24.2 ± 2.2	29.3 ± 3.2	19.7 ± 2.0	17.0 ± 1.8	22.2 ± 2.1	18.3 ± 2.1
4 weeks.....	34.3 ± 3.1	41.5 ± 4.5	30.5 ± 3.1	23.9 ± 2.5	35.4 ± 3.4	33.0 ± 3.8
5 weeks.....	49.4 ± 4.5	61.7 ± 6.8	46.0 ± 4.7	33.7 ± 3.5	48.6 ± 4.6	49.4 ± 5.7
6 weeks.....	63.6 ± 5.8	73.2 ± 8.0	62.7 ± 6.4	45.5 ± 4.7	57.2 ± 5.5	71.4 ± 8.3
8 weeks.....	83.0 ± 7.6	105.3 ± 11.5	85.6 ± 8.7	51.3 ± 5.3	76.4 ± 7.3	86.1 ± 10.0
10 weeks.....	113.7 ± 10.4	142.6 ± 15.6	118.0 ± 12.0	87.7 ± 9.1	96.0 ± 9.2	119.2 ± 13.8
12 weeks.....	138.4 ± 12.7	175.9 ± 19.2	147.5 ± 15.0	104.1 ± 10.8	115.9 ± 11.1	143.6 ± 16.6
16 weeks.....	156.1 ± 14.3	184.2 ± 20.2	165.4 ± 16.8	124.3 ± 12.9	151.9 ± 14.5	167.3 ± 19.4
20 weeks.....	202.6 ± 18.6	215.5 ± 23.6	194.1 ± 19.7	155.7 ± 16.2	199.2 ± 19.0	198.6 ± 23.0
Number of ♀'s.....	27	19	22	21	25	17

TABLE 10

COEFFICIENTS OF VARIATION OF BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS
THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM POISONED
AND OPERATED GROUPS

Age of chicks	Controls	Mercuric chloride	Sodium chloride	Arsenic trioxide	Nicotine sulfate	Yolk removed
Males						
1 day.....	7.6±0.6	7.8±0.8	7.6±0.8	6.7±0.7	8.3±0.9	6.9±0.7
1 week.....	10.4±0.8	16.4±1.8	17.9±1.9	14.1±1.7	11.0±1.2	12.7±1.4
2 weeks.....	15.4±1.2	19.9±2.2	22.4±2.4	17.9±2.1	15.8±1.7	14.4±1.6
3 weeks.....	18.8±1.5	19.4±2.1	23.2±2.5	22.0±2.7	17.6±1.9	16.8±1.8
4 weeks.....	21.0±1.7	20.1±2.2	27.2±3.0	25.8±3.2	19.5±2.1	17.7±1.9
5 weeks.....	21.0±1.7	21.3±2.3	25.7±2.8	26.9±3.3	21.6±2.3	18.6±2.0
6 weeks.....	19.6±1.6	22.8±2.5	25.3±2.8	27.4±3.4	31.1±3.5	19.8±2.2
8 weeks.....	22.5±1.9	24.4±2.7	27.0±3.0	27.0±3.3	22.4±2.4	23.1±2.6
10 weeks.....	20.2±1.6	22.4±2.4	19.2±2.0	27.6±3.4	22.8±2.5	21.0±2.3
12 weeks.....	17.7±1.4	19.2±2.0	17.4±1.8	25.5±3.1	19.1±2.0	20.2±2.2
16 weeks.....	14.3±1.1	14.5±1.5	13.2±1.4	20.4±2.4	15.6±1.7	16.5±1.8
20 weeks.....	12.4±0.9	15.6±1.7	12.2±1.3	19.4±2.3	14.2±1.5	13.5±1.5
Number of ♂s.....	37	21	21	17	21	20
Females						
1 day.....	9.8±0.9	9.3±1.0	8.9±0.9	8.7±0.9	9.2±0.9	8.0±0.9
1 week.....	12.8±1.2	21.0±2.4	14.0±1.5	15.9±1.7	12.9±1.3	11.6±1.4
2 weeks.....	16.7±1.6	24.0±2.8	16.8±1.7	16.7±1.8	16.8±1.7	16.0±1.9
3 weeks.....	21.7±2.1	28.1±3.3	17.6±1.8	17.2±1.8	19.8±1.9	19.2±2.3
4 weeks.....	22.8±2.2	29.7±3.5	19.8±2.1	18.4±1.9	23.0±2.3	23.0±2.0
5 weeks.....	24.3±2.3	31.8±3.8	22.1±2.3	18.4±1.9	23.7±2.4	25.9±3.2
6 weeks.....	23.7±2.3	29.3±3.5	23.0±2.5	18.8±2.0	21.6±2.1	27.6±3.4
8 weeks.....	24.1±2.3	32.6±3.9	24.1±2.6	16.3±1.8	22.1±2.2	25.3±3.1
10 weeks.....	21.7±2.1	30.5±3.6	22.1±2.3	17.6±1.9	18.1±1.8	23.0±2.8
12 weeks.....	19.1±1.8	27.2±3.2	19.9±2.1	15.1±1.6	15.7±1.5	20.2±2.4
16 weeks.....	15.8±1.5	20.5±2.3	16.3±1.7	12.9±1.4	15.4±1.5	17.0±2.0
20 weeks.....	16.5±1.6	19.8±2.2	15.6±1.6	13.3±1.4	16.9±1.7	16.5±2.0
Number of ♀s.....	27	19	22	21	25	17

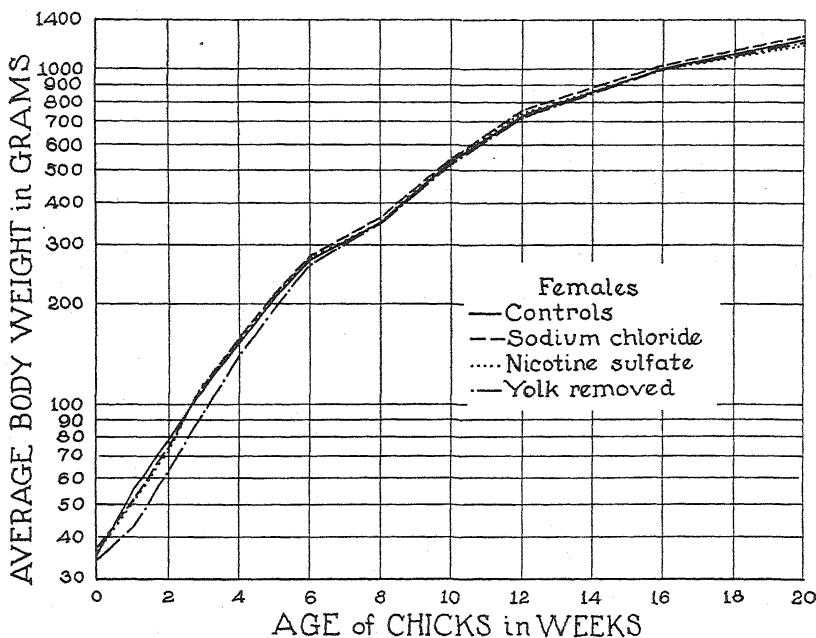


Fig. 8. The average body weights at different ages, of the females that survived the period of 20 weeks, from the operated group, and from groups poisoned by substances normally or frequently administered to chicks.

EXPERIMENTS ON REMOVAL OF THE UNABSORBED YOLK AT ONE DAY OF AGE

Experimental Procedure.—This operation was performed on 64 chicks. Each chick was etherized, an opening about an inch long made in the body wall, the yolk stalk ligatured, the yolk sac and contents completely removed, and the opening sewed up. The operations were performed without aseptic precautions. As soon as the chicks had recovered from the obvious effects of the ether, they were put under the hovers with the other chicks. The mortality was probably higher than would have been the case if more precautions had been taken, but for the purposes of this experiment the high mortality was not disadvantageous, since the questions under investigation were the permanence and selectivity of severe handicaps.

The results obtained in these lots are included with those of the poisoned lots, in tables 8 to 10 and figures 7 and 8.

Effects on Mortality.—As noted previously, in all cases where the treatment of corresponding lots in shipments II and III was exactly comparable,¹¹ the mortality was markedly higher in the lots of shipment III. The difference was significant only in the early mortality, before 3 weeks of age.

Effects on Body Weights of Survivors.—The growth records point to the same result, several indications of which have already been noted; namely, that the females showed more complete recovery from the effect of an early handicap than the males. The difference between the operated males and the control males at 20 weeks of age was probably significant in each shipment, being respectively 144 ± 34 grams and 239 ± 82 grams, with a difference for the combined lots of 137 ± 38 grams. The corresponding differences between the females were not significant at all. The combined lots gave for the females at 20 weeks of age a difference less than the probable error of the difference (22 ± 41 grams); in shipment II the operated females were slightly but not significantly lower than the controls; in shipment III the case was reversed, the operated females being slightly heavier than the controls.

EXPERIMENTS ON EXPOSURE TO EXTREMES OF TEMPERATURE

Although no exact determination of optimum hover temperature for baby chicks has been made, large fluctuations of temperature are avoided in the belief that if the temperature is held for any considerable length of time either too much above or too much below the optimum, the chicks will be injured. The only experimental work which has been encountered (Lewis, 1911) demonstrated an injurious effect from extreme hover temperatures when measured by the resulting mortality. His period of observation was the first 4 weeks of life. The results obtained were as follows:

Hover temperature (F)	Number of chicks at start	Number of chicks that died
110° throughout period.....	50	21
103° first 2 days, reduced $\frac{1}{2}^{\circ}$ every day thereafter.....	50	5
90° first 2 days, reduced $\frac{1}{2}^{\circ}$ every day thereafter.....	50	12
Varying, from 86° to 120°, with average of 102°.....	50	34

¹¹ The treatment of the two shipments was not exactly comparable for the poisoned lots, because of the differences mentioned above, in the age at which the chicks received the poisons, and in point of having had previous feed—and, in the cases of mercuric chloride and arsenic trioxide, in the exact size of the dose.

It therefore seemed of interest to determine whether extreme temperatures maintained for a short time only would produce any effects, on yolk absorption, body weight, and mortality.

Experimental Procedure.—The hover temperature for all the lots where temperature was not the experimental factor was maintained during the first 2 weeks at about 95° F ($=35^{\circ}$ C) with thermostatic control. After the first 2 weeks the hover temperature was gradually lowered, in accordance with the general practice. For the overheated and chilled lots, continuous temperature records were kept by means of thermographs with the sensitive elements under the hover. For the overheated lot of shipment I, the temperature was held at 115° F ($=46.1^{\circ}$ C) for the first 3 nights. Although, as shown in table 12, this did not result in a higher mortality as compared with the controls, the chicks at the time gave every indication of having been injured, remaining at the outermost edge of the hover in an attempt to get away from the heat, panting, and appearing drowsy and generally distressed.

The chicks of shipment IV were given much more severe overheating, the temperature being kept at 125° F ($=51.7^{\circ}$ C) during the first night. As this resulted in no deaths, in the morning the temperature was still further increased, and rose beyond the range of the thermograph. A continuous record of the temperature was therefore not obtained, but readings of chemical thermometers placed under the hover indicated a temperature of about 135° F ($=56.7^{\circ}$ C). Within 2 hours 12 of the 40 chicks were dead, and the temperature was immediately reduced to 100° F ($=37.8^{\circ}$ C). Two more deaths occurred during the next 2 hours, and then only 2 more for the rest of the experimental period of 9 days, one of these being at 4 days and one at 7 days of age.

Similarly with chilling, while the treatment of shipment I was not severe enough to result in a higher mortality than occurred in the controls, it did cause an apparent injury to the chicks at the time. The chilled chicks were entirely deprived of artificial heat for 3 nights, and the windows of the house were left wide open. The temperature in the house outside the hover went to a minimum of 43° F ($=6.1^{\circ}$ C), while the temperature in the midst of the chicks varied between 60° and 70° F ($=15-20^{\circ}$ C). Each morning the chicks were found huddled together in an effort to keep warm, and as a result appeared bedraggled and lacking in vitality.

The chicks of shipment IV were given a slightly more severe treatment, being put in a chick box on the porch on the north side of the laboratory for 2 nights. The temperature reached a minimum of 38° F (=3.3° C) the first night, and of 33° F (=0.6° C) the second night. The temperature in the midst of the chicks reached 55° F (=12.8° C). Six of the 40 chicks died in the chilled group during the experimental period of 9 days, as compared with no deaths in the controls.

TABLE 11

AVERAGE YOLK WEIGHTS (IN GRAMS) OF SAMPLES KILLED AT DIFFERENT AGES,
FROM LOTS EXPOSED TO EXTREMES OF TEMPERATURE, AND
LOTS DEPRIVED OF WATER

Shipment No.	Controls	Overheated	Chilled	Water withheld for 5 days
Chicks killed at 3 days old				
I.....	2.03±0.10	1.84±0.12	2.10±0.22	1.47±0.15
IV.....	2.01±0.18	1.09±0.13	2.27±0.30	1.76±0.22
Total.....	2.02±0.09	1.62±0.11	2.16±0.18	1.59±0.12
Chicks killed at 5 days old				
I.....	0.69±0.09	0.81±0.12	0.71±0.04	1.27±0.15
IV.....	0.82±0.16	0.71±0.17	0.44±0.05	0.83±0.13
Total.....	0.74±0.09	0.79±0.10	0.61±0.03	1.09±0.11
Chicks killed at 7 days old				
I.....	0.28±0.06	0.18±0.04	0.38±0.08	0.56±0.14
IV.....	0.09±0.04	0.27±0.10	0.20±0.04	0.23±0.03
Total.....	0.20±0.04	0.20±0.04	0.28±0.05	0.50±0.12
Chicks killed at 9 days old				
I.....	0.06±0.03	0.00±0.02	0.06±0.02	0.07±0.04
IV.....	0.22±0.06	0.09±0.03	0.05±0.01	0.02±0.01
Total.....	0.13±0.03	0.03±0.01	0.05±0.01	0.06±0.03

Effects on Yolk Absorption.—From the averages given in table 11 and plotted in figure 9, it appears that overheating caused some acceleration in yolk absorption, and chilling a slight retardation, but only during the period of actual exposure to temperatures above or below the optimum. In all four samples killed at 3 days of age, differences were found which, although with one exception not significant in themselves, were all consistent as to direction, the overheated chicks

having smaller yolks, and the chilled chicks larger yolks, than the controls. The difference between the average yolk weights of the overheated and control groups of shipment I is not at all significant, being very little larger than the probable error of the difference. The corresponding difference in shipment IV, however, is more than four times the probable error of the difference (0.92 ± 0.22 grams). In connection with this it is to be remembered that the chicks of shipment IV were subjected to a more severe overheating than those of

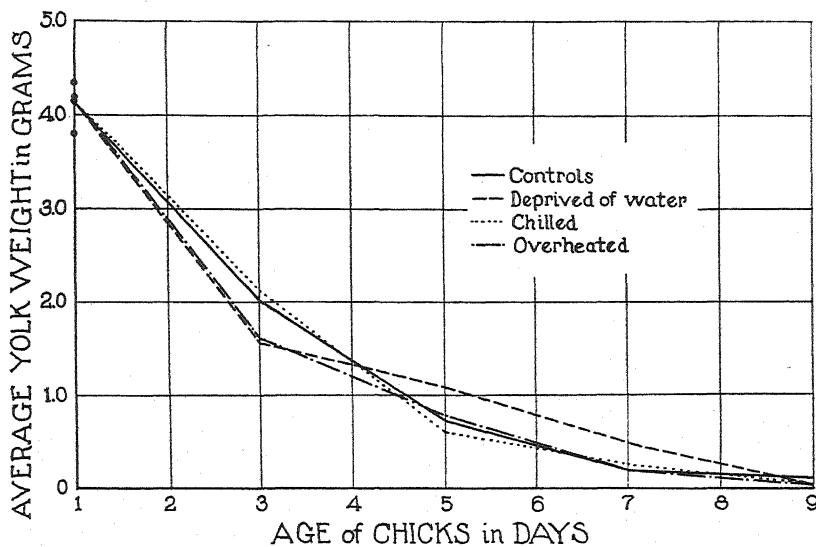


Fig. 9. The average yolk weights of chicks killed at different ages, from groups exposed to extremes of temperature, and from the group deprived of water.

shipment I. In the chilled groups, the differences in both shipments were small, and of no significance in comparison with their probable errors.

The averages at later ages showed no evidence of any permanent effect upon yolk absorption from early exposure to extreme temperatures.

Effects on Mortality.—As previously indicated, the overheating and chilling which the lots of shipment I received did not result in a higher mortality than occurred in the controls. As with the other handicaps discussed so far there was no uniformity in the comparative mortality rates of the two sexes. The rates are shown in table 12.

TABLE 12

MORTALITY RATES (PER 100) OF LOTS EXPOSED TO EXTREMES OF TEMPERATURE AND LOT DEPRIVED OF WATER

Designation	Controls	Overheated	Chilled	Water withheld for 5 days
Total mortality (through 20 weeks).....	12.9±2.7	9.1±2.3	12.3±2.6	11.4±2.5
Early mortality (for first 3 weeks).....	4.3±1.6	3.5±1.5	2.4±1.2	8.5±2.2
Later mortality (3 wks. through 20 wks.)	8.6±2.2	5.6±1.9	9.9±2.4	2.9±1.4
Total mortality (through 20 weeks) ♂s....	17.3±4.0	14.2±4.0	7.9±2.9	13.1±4.1
Total mortality (through 20 weeks) ♀s....	7.0±3.1	4.5±2.1	17.3±4.3	10.1±3.2

TABLE 13

AVERAGE BODY WEIGHT AT DIFFERENT AGES, OF THE CHICKS THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM LOTS EXPOSED TO EXTREMES OF TEMPERATURE AND LOT DEPRIVED OF WATER

Age of chicks	Controls	Overheated	Chilled	Water withheld for 5 days
Males				
1 day.....	37.3± 0.3	38.2± 0.4	37.4± 0.3	37.5± 0.4
1 week.....	56.3± 0.8	58.0± 0.9	54.1± 0.7	48.5± 0.8
2 weeks.....	82.6± 1.7	87.0± 2.0	80.1± 1.3	76.3± 1.7
3 weeks.....	120.2± 2.7	123.2± 3.4	117.4± 2.1	114.6± 3.0
4 weeks.....	168.0± 4.0	174.5± 5.3	167.0± 3.3	165.8± 4.6
5 weeks.....	225.7± 5.2	238.2± 7.0	229.8± 4.7	227.0± 6.4
6 weeks.....	286.1± 6.8	295.2± 8.5	288.5± 5.6	285.5± 8.6
8 weeks.....	393.9±10.3	412.5±10.1	406.2± 7.6	392.1±12.1
10 weeks.....	609.2±12.2	643.8±14.9	640.0± 9.7	615.8±16.9
12 weeks.....	803.8±14.0	847.9±15.7	846.3±11.1	806.9±17.9
16 weeks.....	1,220.3±19.0	1,289.3±20.5	1,296.3±16.8	1,209.2±25.3
20 weeks.....	1,510.3±26.3	1,601.0±23.5	1,609.4±19.2	1,514.6±26.6
Number of ♂s.....	32	29	35	26
Females				
1 day.....	37.3± 0.3	37.2± 0.3	37.3± 0.4	37.6± 0.4
1 week.....	56.0± 1.0	56.2± 0.7	54.6± 0.7	48.6± 0.7
2 weeks.....	81.0± 1.8	82.4± 1.6	77.9± 1.6	73.3± 1.3
3 weeks.....	112.9± 2.7	114.2± 2.8	113.6± 2.5	104.5± 2.5
4 weeks.....	154.7± 4.3	160.4± 4.4	159.8± 3.7	151.2± 3.9
5 weeks.....	204.4± 5.6	219.3± 6.3	219.6± 5.2	201.7± 5.9
6 weeks.....	257.9± 7.1	272.9± 8.2	277.9± 7.3	258.5± 7.8
8 weeks.....	361.6± 9.3	374.6±11.1	380.0±10.4	352.1±11.2
10 weeks.....	545.7±10.8	568.3±14.1	588.9±11.5	545.9±13.4
12 weeks.....	736.1±13.5	762.2±16.7	771.9±14.9	734.7±15.7
16 weeks.....	1,016.4±16.8	1,048.1±17.9	1,050.4±18.3	1,030.3±16.9
20 weeks.....	1,225.4±18.9	1,276.1±21.2	1,240.4±23.7	1,252.1±20.7
Number of ♀s.....	28	36	27	34

Effects on Body Weights of Survivors.—It is apparent from the averages presented in table 13 and plotted in figures 10 and 11 that neither the overheating nor the chilling effected any significant differences in body weights for either sex or at any age. Among the males, the overheated lots at all ages, and the chilled lots at all ages after 5 weeks were heavier, but not significantly heavier, than the controls. Similarly the females in the overheated lot were slightly heavier at all ages, and in the chilled lots at all ages after 3 weeks.

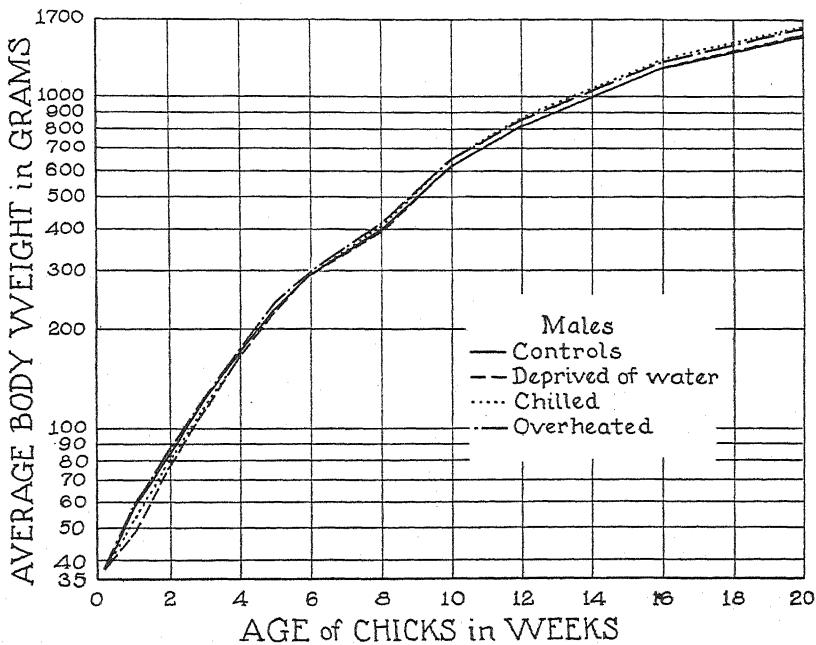


Fig. 10. The average body weights at different ages, of the males that survived the period of 20 weeks, from groups exposed to extremes of temperature, and from the group deprived of water.

In the light of these results it is perhaps unfortunate that the early injury was not made more severe, but at the time it seemed more valuable to determine whether individuals which appeared injured would completely recover, than to show that they could be so severely injured that they would not recover. Showing this in groups where the mortality was not higher than in the controls has the added advantage of showing that later recovery does not necessarily depend upon a selective effect of the mortality, by an elimination of the poorer individuals.

There is only one point worthy of especial mention in connection with the standard deviations and coefficients of variation presented in tables 14 and 15. The control females of this shipment showed a relative variability slightly less than that of the corresponding group of males, whereas, in all other data analyzed so far, the sex difference in relative variability has been the reverse. The difference in this case is, however, absolutely insignificant.

TABLE 14

STANDARD DEVIATIONS OF BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS
THAT SURVIVED THE PERIOD OF 20 WEEKS FROM LOTS EXPOSED
TO EXTREMES OF TEMPERATURE AND LOT
DEPRIVED OF WATER

Age of chicks	Controls	Overheated	Chilled	Water withheld for 5 days
Males				
1 day.....	2.4± 0.2	3.1± 0.3	2.9± 0.2	2.8± 0.3
1 week.....	6.8± 0.6	7.2± 0.6	6.1± 0.5	5.8± 0.6
2 weeks.....	14.1± 1.2	15.9± 1.4	11.1± 0.9	13.2± 1.2
3 weeks.....	22.8± 1.9	27.2± 2.4	18.2± 1.5	22.4± 2.1
4 weeks.....	33.5± 2.8	42.1± 3.7	29.3± 2.4	34.8± 3.3
5 weeks.....	44.0± 3.7	56.1± 5.0	41.6± 3.4	48.8± 4.6
6 weeks.....	57.3± 4.8	67.7± 6.0	48.8± 3.9	64.7± 6.0
8 weeks.....	86.7± 7.3	80.4± 7.1	66.6± 5.4	91.3± 8.5
10 weeks.....	102.4± 8.6	118.7± 10.5	85.1± 6.9	127.5± 11.9
12 weeks.....	117.7± 9.9	125.4± 11.1	97.2± 7.8	135.4± 12.7
16 weeks.....	161.1± 13.6	163.9± 14.5	145.2± 11.7	191.5± 17.9
20 weeks.....	220.4± 18.6	187.6± 16.6	168.4± 13.6	201.0± 18.8
Number of ♂'s.....	32	29	35	26
Females				
1 day.....	2.6± 0.2	2.7± 0.2	3.1± 0.3	3.1± 0.3
1 week.....	8.0± 0.7	6.7± 0.5	5.0± 0.5	6.0± 0.5
2 weeks.....	13.8± 1.2	14.6± 1.2	12.1± 1.1	11.4± 0.9
3 weeks.....	21.4± 1.9	25.0± 2.0	19.5± 1.8	21.7± 1.8
4 weeks.....	34.1± 3.1	38.7± 3.1	28.7± 2.6	33.9± 2.8
5 weeks.....	44.3± 4.0	55.8± 4.4	40.4± 3.7	50.6± 4.1
6 weeks.....	55.4± 5.0	72.8± 5.8	56.0± 5.1	67.2± 5.5
8 weeks.....	72.7± 6.6	99.1± 7.9	79.9± 7.3	96.6± 7.3
10 weeks.....	85.0± 7.7	125.1± 9.9	88.4± 8.1	116.2± 9.5
12 weeks.....	105.6± 9.5	148.5± 11.8	115.0± 10.6	135.7± 11.1
16 weeks.....	132.0± 11.9	159.6± 12.7	140.8± 12.9	146.3± 12.0
20 weeks.....	148.1± 13.3	188.3± 15.0	182.5± 16.8	178.9± 14.6
Number of ♀'s.....	28	36	27	34

TABLE 15

COEFFICIENTS OF VARIATION OF BODY WEIGHTS AT DIFFERENT AGES, OF THE CHICKS THAT SURVIVED THE PERIOD OF 20 WEEKS, FROM LOTS EXPOSED TO EXTREMES OF TEMPERATURE AND LOT DEPRIVED OF WATER

Age of chicks	Controls	Overheated	Chilled	Water withheld for 5 days
Males				
1 day.....	6.4±0.5	8.1±0.7	7.8±0.6	7.5±0.7
1 week.....	12.1±1.0	12.4±1.1	11.3±0.9	12.0±1.1
2 weeks.....	17.1±1.5	18.3±1.6	13.0±1.2	17.3±1.7
3 weeks.....	19.0±1.7	22.1±2.0	15.5±1.3	19.5±1.9
4 weeks.....	19.9±1.8	24.1±2.2	17.5±1.5	21.0±2.0
5 weeks.....	19.5±1.7	23.6±2.2	18.1±1.5	21.5±2.1
6 weeks.....	20.0±1.8	22.9±2.1	16.9±1.4	22.7±2.2
8 weeks.....	22.0±1.9	19.5±1.8	16.4±1.4	23.3±2.3
10 weeks.....	16.8±1.4	18.4±1.6	13.3±1.1	20.7±2.0
12 weeks.....	14.6±1.2	14.8±1.3	11.5±0.9	16.8±1.6
16 weeks.....	13.2±1.1	12.7±1.1	11.2±0.9	15.8±1.5
20 weeks.....	14.6±1.2	11.7±1.0	10.5±0.8	13.3±1.2
Number of ♂'s.....	32	29	35	26
Females				
1 day.....	7.0±0.6	7.3±0.6	8.3±0.7	8.2±0.7
1 week.....	14.3±1.3	11.9±1.0	9.2±0.8	12.3±1.0
2 weeks.....	17.0±1.6	17.7±1.4	15.5±1.5	15.6±1.3
3 weeks.....	19.0±1.8	21.9±1.8	17.2±1.6	20.8±1.8
4 weeks.....	22.0±2.1	22.2±1.9	18.0±1.7	22.4±1.9
5 weeks.....	21.7±2.1	25.4±2.1	18.4±1.7	25.1±2.2
6 weeks.....	21.5±2.0	26.7±2.3	20.2±1.9	26.0±2.3
8 weeks.....	20.1±1.9	26.5±2.3	21.0±2.0	27.4±2.4
10 weeks.....	15.6±1.4	22.0±1.8	15.0±1.4	21.3±1.8
12 weeks.....	14.3±1.3	19.5±1.6	14.9±1.4	18.5±1.6
16 weeks.....	13.0±1.2	15.2±1.2	13.4±1.2	14.2±1.2
20 weeks.....	12.1±1.1	14.8±1.2	14.7±1.4	14.3±1.2
Number of ♀'s.....	28	36	27	34

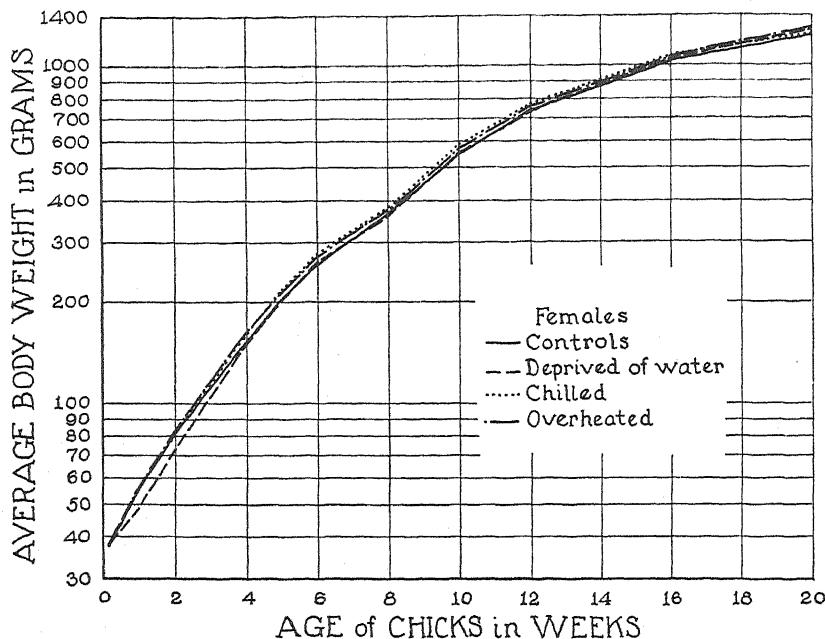


Fig. 11. The average body weights at different ages, of the females that survived the period of 20 weeks, from groups exposed to extremes of temperature, and from the group deprived of water.

EXPERIMENTS ON DEPRIVATION OF WATER

Experimental Procedure.—Preliminary trials had indicated that chicks could be deprived of water for about 5 days. Autopsies showed shriveled intestines, which were not, however, black as in the cases of completely starved chicks. Moreover the skin and mesenteries appeared extremely dry. This period of 5 days was used therefore for one lot of shipment I. Recovery was so immediate and complete, after water was given to the surviving chicks on the fifth day, that the total mortality for the lot was no greater than that of the controls. Therefore in the corresponding lot of shipment IV water was withheld one additional day. Fourteen of the 40 chicks of this lot died, 1 at 5, 9 at 6, 3 at 7, and 1 at 8 days of age, as compared with no deaths among the controls.

The results are included with those of the overheated and chilled groups, in tables 11 to 15 and figures 9 to 11.

Effects on Yolk Absorption.—The chicks deprived of water had, on the average, smaller yolks at 3 days of age than the corresponding control lots. While the difference between the handicapped and control lots of shipment I appears to be significant (0.56 ± 0.18 grams), between the corresponding lots of shipment IV the difference is less than the probable error of the difference (0.25 ± 0.29 grams). When the data from the two shipments are combined, the difference is therefore not significant (0.43 ± 0.18 grams).

In the samples of chicks killed at later ages there were no consistent differences, in spite of the fact that in both shipments the handicap was still being applied up to 5 days of age. The chicks of shipment I which had no water averaged markedly larger yolks than the controls (difference = 0.58 ± 0.18 grams), but those of shipment IV averaged the same as the controls. The evidence, therefore, was not very convincing even as to the immediate effect of deprivation of water upon yolk absorption, and the results show clearly that there was no permanent effect, the samples at 7 and 9 days of age showing no consistent differences as compared with the controls.

Effects on Mortality.—These data have already been discussed in connection with the experimental procedure.

Effects on Body Weights of Survivors.—Both males and females of the lot deprived of water were, at 1 week of age, significantly below the control lot in body weight. This difference was effaced gradually. In both sexes, at about 4 weeks of age the handicapped and control chicks became practically identical in average weights, and remained so for the duration of the experiment.

SUMMARY OF EFFECTS OF EARLY HANDICAPS

From the foregoing detailed examination of the results of the various handicaps the following facts seem to emerge. Yolk absorption, although highly variable within any group of chicks, is after all a relatively stable process, not easily affected by such experimental treatment as were employed. In some cases the latter were so drastic as to result in mortality rates as high as 70 per cent. Even in these cases, however, no large differences in the course of yolk absorption were evident.

Similarly with the effects on body weights, with the varied and severe experimental handicaps, there were few groups in which the average weight of the experimental groups did not equal that of the

controls by the age of 20 weeks. In most of the groups complete recovery was shown at 6 weeks of age. In the females the only group which did not show complete recovery was that given mercuric chloride. In the males, there were indications of more permanent effects from several of the handicaps, not significant in the starved groups, but probably significant in the cases of mercuric chloride, nicotine sulfate, yolk removal, and the larger dose of arsenic trioxide.

ANALYSIS OF COMBINED DATA FROM ALL EXPERIMENTS

Certain interesting relationships are brought out by consideration of the combined data of all the experiments. These include the comparisons of mortality rates by sex, comparisons of chicks which died with those which survived, and correlations of day-old body weights with yolk weights, and with later body weights.

Mortality Rates by Sex.—It has been noted already, in connection with the discussion of the various handicaps separately, that there was no apparent differential sex effect in mortality, although several indications were found of a differential effect in body weight (more permanent effect in males). It was thought that by combining the data of the several experiments such a differential effect in mortality might appear. Therefore the sex mortality rates for all the chicks, and for several major groupings, were calculated, with the results shown in table 16.

No difference was found between the male and female mortality for the total group of chicks, the respective rates being 21.8 and 21.0. Dividing the data into three groups corresponding roughly to the severity of the treatment, as severely handicapped, moderately handicapped, and controls, the male and female rates were still practically identical. This was true both when the total mortality to 20 weeks was considered, and when early and later mortality were treated separately.

Early and Late Mortality.—The division into early and late mortality demonstrated further that all the increase in mortality in the severely handicapped groups was evidenced early. There was no evidence that the later mortality of the experimentally injured groups was either higher than the controls, as would result from a permanent injury and increased susceptibility to later chance hazards, or that it was lower than the controls, as might be expected if the early elimination had acted selectively.

TABLE 16

MORTALITY RATES (PER 100) BY SEX, FOR THE ENTIRE AGGREGATE OF CHICKS, AND FOR CERTAIN MAJOR GROUPINGS

Designation	Total mortality (through 20 weeks)		
	Males	Females	Total
Controls.....	13.3±2.5	11.2±2.7	12.4±1.8
Moderately handicapped.....	11.7±1.5	12.7±1.6	12.2±1.1
Severely handicapped.....	38.9±2.5	37.1±2.3	37.9±1.7
Total.....	21.8±1.3	21.0±1.2	21.4±0.9
Mortality rates (for first 9 days)			
Controls.....	4.7±1.5	3.2±1.2	4.1±1.1
Moderately handicapped.....	4.5±0.9	6.5±1.1	5.6±0.8
Severely handicapped.....	29.6±2.2	30.5±2.2	30.0±1.6
Total.....	13.5±1.1	14.4±1.1	14.0±0.8
Mortality rates (10 days through 20 weeks)			
Controls.....	8.6±2.1	8.0±2.4	8.3±1.5
Moderately handicapped.....	7.2±1.2	6.2±1.1	6.6±0.9
Severely handicapped.....	9.3±1.4	6.6±1.1	7.9±0.9
Total.....	8.3±0.9	6.6±0.8	7.4±0.6

Comparison of Day-Old Body Weights of Chicks That Died With Those of Chicks That Survived.—Evidence that the early mortality was to a certain extent selective was however obtained from a comparison of the day-old body weights of the chicks which succumbed with the day old body weights of those which survived. The average weights and frequency distributions are presented in table 17.

The data show that, among the males and females alike, the chicks that subsequently died were, at 1 day of age, on the average significantly smaller than the chicks that survived. Further, the chicks that died at ages of 10 days or older were intermediate in day-old weights, between those that died before 10 days of age and those that survived the entire period of 20 weeks.

Finally, the results show that the selective effect was slightly greater among the males than among the females, the difference between the average weights of the cockerels that survived and those that died before 10 days being 1.88 ± 0.20 grams, while the corresponding difference for the pullets was 1.22 ± 0.21 grams. For the cockerels that died before 10 days of age, the average weight was actually slightly less than that of the pullets, so that the sex difference was the reverse of that ordinarily found.

TABLE 17

FREQUENCY DISTRIBUTIONS OF DAY-OLD BODY WEIGHTS OF CHICKS THAT DIED,
COMPARED WITH THOSE OF CHICKS THAT SURVIVED.
(SHIPMENTS I-III)

Day-old body weight (grams)	Males			Females		
	Died before 10 days	Died, 10 days -20 weeks	Survived	Died before 10 days	Died, 10 days -20 weeks	Survived
30	2	2	4	2	3
31	1	1	4	2	9
32	7	5	15	10	3	34
33	13	1	7	6	20
34	15	4	37	17	5	37
35	15	3	50	19	5	41
36	12	7	37	16	2	42
37	8	4	37	8	4	33
38	6	6	49	6	4	40
39	6	4	31	5	2	31
40	3	1	31	4	4	34
41	9	1	7
42	1	17	13
43	2	1	2
44	1	1	1	4
45	2	1	4
46	1	1
47
48
49	1
Total number of chicks.....	88	38	333	100	32	355
Average body weight.....	35.10±0.17	36.18±0.31	36.98±0.11	35.27±0.18	36.09±0.37	36.49±0.11

TABLE 18

AVERAGE YOLK WEIGHTS OF CHICKS THAT DIED, IRRESPECTIVE OF CAUSE OF DEATH,
COMPARED WITH YOLK WEIGHTS OF CHICKS KILLED
AT CORRESPONDING AGES

Age of chicks (in days)	Yolk weights of chicks that died	Yolk weights of chicks killed		
		Males and females	Total	Males
1	4.68±0.29	4.54±0.06	4.42±0.08	4.68±0.08
2	3.50±0.14
3	2.50±0.08	2.00±0.06	1.98±0.08	2.02±0.08
4	2.51±0.16
5	1.93±0.24	0.82±0.03	0.69±0.04	0.94±0.05
6	0.93±0.12
7	1.06±0.14	0.31±0.02	0.27±0.03	0.36±0.04
8	0.97±0.21
9	1.68±0.39	0.21±0.03	0.20±0.05	0.21±0.04

Comparison of Yolk Weights of Chicks That Died with Those of Samples Killed at Corresponding Ages.—Chicks that died, irrespective of the cause of death, showed significant differences in yolk weights from the chicks that were killed at corresponding ages. The averages are presented in table 18 and the frequency distributions in table 19. The averages are plotted in figure 12.

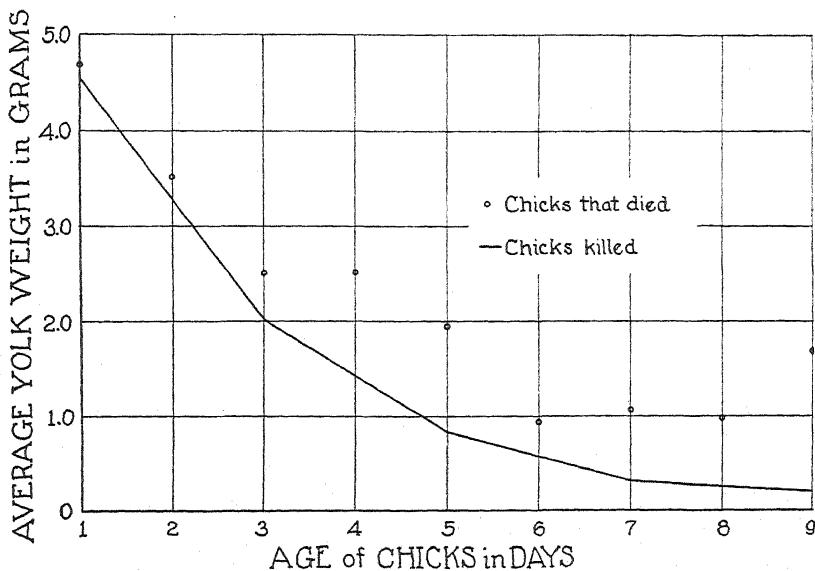


Fig. 12. The average yolk weights of chicks that died, compared with those of chicks killed at corresponding ages.

It is apparent that the chicks that died had on the average larger yolks than those killed at corresponding ages, and that the differences increased with age. These results are quite in accordance with what would be expected, namely, that any disturbance of normal functioning severe enough to cause death would be likely to interfere with the normal metabolism of the yolk.

That it does not always do so, however, is evident from an examination of the frequency distributions in table 20. These tabulations show that many of the chicks that died had yolks of normal size for their age, as compared with samples killed. Conversely, a few of the chicks that were killed, and apparently perfectly normal, had yolks as large as, or larger than, any found in the chicks dying at corresponding ages.

TABLE 19

FREQUENCY DISTRIBUTIONS OF YOLK WEIGHTS OF CHICKS THAT DIED, COMPARED
WITH THOSE OF CHICKS KILLED AT CORRESPONDING AGES

Yolk weight (grams)	Age of chicks (in days)													
	1		2		3		4		5		6			
	Died	Killed	Died	Died	Died	Killed	Died	Died	Died	Killed	Died	Died		
0.00-0.009					1				4		3	61		
0.01-0.09									17	5	5	83		
0.10-0.19									20	2	1	11		
0.20-0.29								1	27	2	1	14		
0.30-0.39					2		3	30	3			13		
0.40-0.49			1	4			6	44	4	1	6			
0.00-0.49			2	6		10	142	16	11	188	8	4		
0.50-0.99			1	2	37	4	3	72	8	6	33	2		
1.00					4	51	8	2	23	4	7	16		
1.50		4	6	7	44		9	4	14	5	4	2		
2.00		11	11	11	35		2	2	17		1	1		
2.50	1	7	11	15	24		5	1	3		1	2		
3.00		28	7	7	16		5		3	1	1			
3.50		44	7	5	7		3	4	3					
4.00		2	41	5	2	3	1				1	1		
4.50		2	39	5	1	4		1	2		1			
5.00	1	19	1				3	1	1			1		
5.50		25	2		1					1				
6.00		10	2		1							1		
6.50	1	12	2		1									
7.00		3												
7.50		3			1	1								
8.00		2					1							
8.50														
9.00														
9.50														
10.00			1											
10.50				1										
11.00					1									
11.50-11.99						1								
Total number of chicks.	7	250	61	56	232	41	29	280	35	33	243	16	10	227

The correlation between general vigor and yolk absorption indicated by the higher average yolk weights of the chicks that died in comparison with the killed chicks was evidenced also *within* the samples killed, on correlating the percentage gain in body weight with the weight of the unabsorbed yolk. Among the 38 control chicks killed at 5 days old this correlation amounted to $-0.31 \pm .10$, and among the 36 control chicks killed at 7 days old, to $-0.38 \pm .10$. Stating it in another way: of the chicks killed at 5 days old, those with yolks weighing less than 0.50 grams had gained 20 per cent of their body weight; those with yolks weighing more than 0.50 grams had gained 11 per cent of their body weight. In the chicks killed at 7 days old, those with yolks weighing less than 0.10 grams had gained 31 per cent of their body weight, while chicks with yolks weighing more than 0.10 grams had gained only 16 per cent of their body weight.

These results are contrary to the conclusion reached by Schilling and Bleeker (1928), who state that "there is no indication in this group of chicks that chicks making more rapid gains used up their yolk more rapidly or that slow absorption was accompanied by retarded gains." Their conclusion was reached, however, by a comparison of the body weights of particular individuals at the time they were killed, and without reference to the hatching weights of the same birds. Our experience agrees with theirs in finding individuals furnishing marked exceptions to a direct relationship between rapid gains and rapid yolk absorption; in fact, the low values of the correlation coefficients indicate in themselves that such would be the case. The fact seems worthy of note, however, and of further confirmation, that a significant correlation can be demonstrated. It should be emphasized that this correlation furnishes no evidence for the *causal* relation between yolk absorption and general vigor so commonly assumed. It indicates only that both processes are subject to some common influences, or as stated above, any disturbance of normal functioning is likely to affect both yolk metabolism and general metabolism. Experiments designed to serve as more crucial tests of these particular points are contemplated.

Occasional chicks were found with yolks abnormal in other respects than that of size. Some were putrified, some hardened, and a few bloody. None of these abnormal appearances could be shown to have any relationship to the treatment which the chicks had received. They were found occasionally in all lots, and not only in chicks that died, but also in some which had been killed and were apparently normal.

Whether the killed chicks which showed these abnormalities would have died subsequently it is of course impossible to state.

Some related observations from preliminary experiments are of interest in this connection. While considering the possibility of making repeated observations on the course of yolk absorption in the same chicks, by successive operations and estimation of the amount of yolk present, a few abnormally large, and a few hardened yolks were found. These chicks were able to survive both the effects of the successive operations and the presence of the unabsorbed yolks throughout the period of the preliminary experiments. It is hoped that these experiments can be repeated on a more extensive scale.

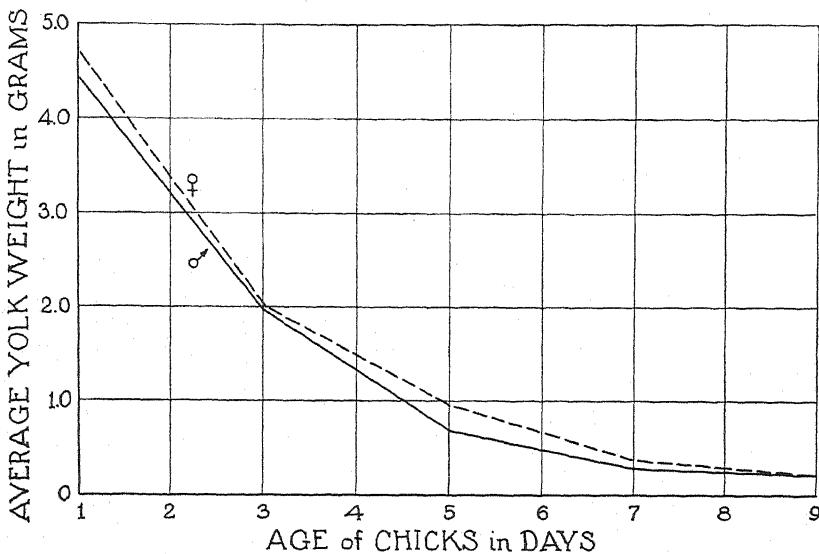


Fig. 13. The average yolk weights at different ages, of males and females separately.

In table 18 are included also the yolk weights for the two sexes separately, for the samples killed at different ages. These averages are plotted in figure 13. The fact that the females averaged slightly heavier yolk weights at every age is interesting, particularly in connection with the smaller average body weight of the females, and the positive correlation between day-old body weights and yolk weights at different ages, discussed below.

Correlation Between Day-Old Body Weights and Yolk Weights at Different Ages.—Mention was made above of the fact that an essential precaution in selecting samples of chicks to kill for yolk weights

at different ages was to make, not only the average day-old body weight, but also the frequency distribution of day-old body weights, of the different samples the same. Without this precaution, comparisons could not justifiably be made between the different lots at a given age, or between the samples at different ages within a given experimental lot, as is evidenced by the correlation between day-old body weights and the yolk weights of the chicks killed at various ages.

The correlation coefficients between day-old body weight and weight of unabsorbed yolk, in chicks killed at various ages, were as follows:

Age of chicks	Correlation coefficient
1 day.....	+0.48±0.03
3 days.....	+0.34±0.04
5 days.....	+0.13±0.04
7 days.....	+0.02±0.04
9 days.....	+0.08±0.04

While perhaps it is not surprising that there was a significant positive correlation between the body weight of the chick and the weight of the yolk at 1 day old, it was surprising to find how long the correlation persisted. It was still significant in the chicks killed at 5 days of age.

These correlations have been worked out also for the two sexes separately and for certain of the shipments separately. The results were in all cases substantially the same.

It was also interesting to note the steepness of the slopes of the regression lines, when average yolk weight was plotted against the day-old body weight. These lines are shown in figure 14. For the chicks killed at 1 day old and at 3 days old, not only were the *absolute* yolk weights on the average larger in the larger chicks, but also the *percentage* yolk weights were considerably higher in the larger chicks. The dotted lines indicate the relation which would exist if the percentage yolk weight were constant for chicks of different body weights, and equal to the average for the entire group. (The average yolk weight for chicks killed at 1 day of age was 12.5 per cent of the day-old body weight, and for chicks killed at 3 days of age, 5.3 per cent of the day-old body weight.)

Since, as a number of investigators have found (see Curtis, 1914, and Atwood and Weakley, 1917), the percentage of yolk in unincubated eggs is slightly lower in the heavier eggs, these results would appear to indicate that of the total yolk present at the beginning of development, relatively less of it is utilized in the course of development of the smaller chicks than in the case of the larger.

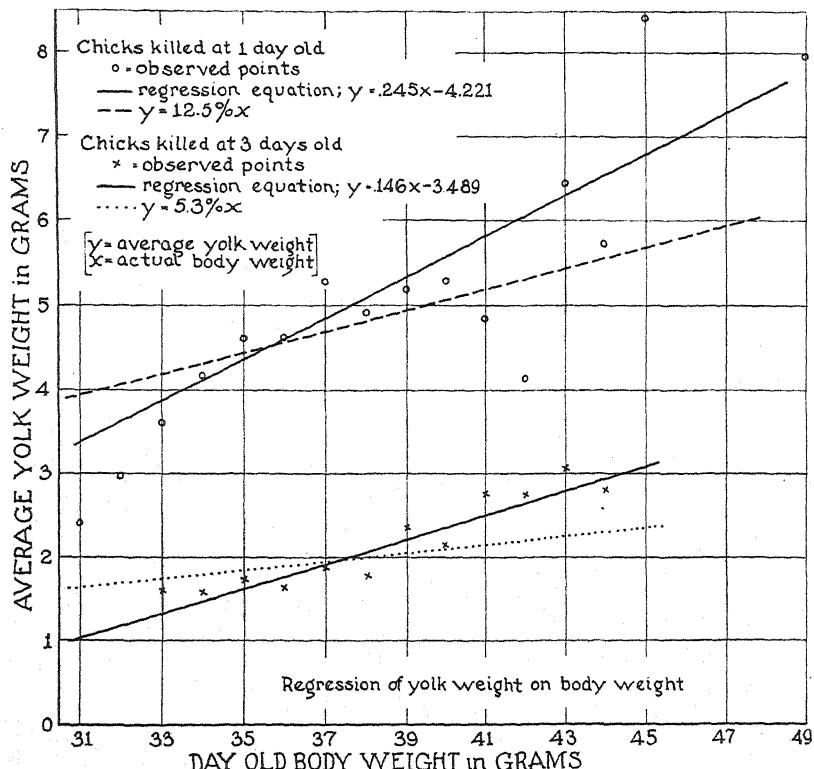


Fig. 14. The regression of yolk weight on day-old body weight, for chicks killed at 1, and at 3 days of age.

Correlation Between Day-Old Body Weights and Later Weights.—While day-old body weight of the chicks proved to be an important factor in equalizing the different experimental groups at the start, because of its correlation with yolk weights and its connection with survival value, it did not appear to be of importance in relation to the later body weights of the chicks. For the controls, the correlations of day-old weights with final weights at 20 weeks were practically zero for both males ($+0.04 \pm 0.08$) and females ($+0.08 \pm 0.09$). For the entire aggregate of chicks, the correlations were on the border line of significance ($+0.12 \pm 0.04$ and $+0.11 \pm 0.04$).

The correlations were worked out separately for the different shipments, with the following results:

Shipment	Correlation coefficient	
	Males	Females
I.....	$+0.24 \pm 0.06$	$+0.06 \pm 0.06$
II.....	$+0.004 \pm 0.061$	$+0.19 \pm 0.06$
III.....	$+0.15 \pm 0.07$	$+0.11 \pm 0.06$

There was thus indication of a significant correlation between day-old weights and later weights only in shipment I in the case of the males, and in shipment II in the case of the females.

Correlations for still other groupings, and for other ages, have been worked out, but those presented are sufficient to demonstrate the almost complete lack of correlation between the day-old weights and later weights. This was apparent in all series, and seems to indicate that early inequalities producing variations in size of day-old chicks (such as size of egg, etc.) were in general without permanent effect on body weight. This result is consistent with the conclusion reached from the analysis of the results of subjection of chicks to experimental handicaps, that such chicks in most cases showed complete recovery by 20 weeks of age, when judged by average body weight in comparison with controls.

CONCLUSIONS

1. A variety of early handicaps including poisons, starvation, and high and low temperatures, failed to alter markedly the course of yolk absorption in baby chicks.
2. The same series of early handicaps, and the removal of the unabsorbed yolk when chicks were 1 day of age, failed in general to produce permanent effects in body weights.
3. The doses of mercuric chloride seemed to show a more permanent effect on body weight than the other handicaps, both males and females so treated weighing less at 20 weeks of age than the controls.
4. Nicotine sulfate, the larger dose of arsenic trioxide, and the removal of unabsorbed yolk when chicks were 1 day old, all tended to show permanent effects in males, but not in females. Starvation also gave indications of a more lasting effect in the males, but the effect was not found in all shipments.
5. No consistent correlations appeared between day-old body weights and body weights at 20 weeks of age.
6. There was a significant positive correlation between day-old body weight and the weight of unabsorbed yolk, up to and including the age of 5 days.
7. The mortality of chicks up to the age of 20 weeks was selective in respect to day-old body weights, tending to eliminate the smaller chicks. The early mortality was more stringently selective than the later.
8. Chicks that died, irrespective of cause, had on the average larger unabsorbed yolks than survivors killed at corresponding ages. This difference increased with age during the 9-day period of observation.
9. Among the chicks killed at a particular age, there was found to be a low but significant correlation between yolk absorption and the percentage gain in body weight.
10. The occasional abnormal yolks found, including putrified, hardened, and bloody yolks, could not be shown to have any relationship to the treatment which the chicks had received.

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APPENDIX

RAW DATA

TABLE 21
REDUCED TABLE OF RAW DATA ON MORTALITY

15

TABLE 29
REDUCED TABLE OF RAW DATA ON BODY WEIGHTS AT DIFFERENT AGES, OF THE MALES WHICH SURVIVED THE EXPERIMENTAL PERIOD OF 20 WEEKS

TABLE 23
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CHANGES IN THE NITRATE AND SULFATE CONTENT OF THE SOIL SOLUTION UNDER ORCHARD CONDITIONS

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In 1922, the Division of Pomology of the California Agricultural Experiment Station began an investigation on the maintenance of soil fertility in deciduous orchards. For this purpose a field of approximately twelve acres was selected and half of it planted to deciduous fruit trees. This will hereafter be referred to as block *A*. The following year, 1923, the other half was set out. It will be designated block *B*. The soil in the field selected varied from a fine sandy loam to a loam of the Yolo series. The water table stood at approximately sixteen feet. The entire volume of soil above the water table was in horizon *A*. There were some slight modifications in texture before that depth was reached, but neither gravel nor clay was found.

CROP HISTORY OF THE FIELD USED

The crop history of this field before the planting of the orchard is of interest.† In the summer of 1908, this area was levelled for alfalfa irrigation. Previous to this time it had been in grain for an indefinite period, probably about fifty years. In the spring of 1909, it was planted to alfalfa. From 1909 to 1913, there is no available record as to yields or irrigation frequency, but it is supposed that the entire area had essentially the same treatment.

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† Professor S. H. Beckett of the Division of Irrigation Investigations and Practice has supplied the data for this section.

In 1914, 5.12 acre feet per acre of irrigation water were applied, distributed among three irrigations. Five crops of hay were cut, with an average yield of 6.45 tons per acre. In 1915, one irrigation of 1.7 acre feet per acre was given. The average yield was 5.04 tons per acre. In 1916, the field was plowed in the spring and seeded to barley, but not irrigated. The yield was about fifty bushels per acre. In June, after the grain had been harvested, the field was irrigated and planted to corn for ensilage. Because of late seeding, only three tons per acre were obtained. In 1917, the area was planted to corn (variety tests) and forage crops in small plots. Irrigation was uniform for the block, a total of 1.21 acre feet per acre being applied in two irrigations.

In 1918, alfalfa was again planted and irrigated once. In 1919, 2.3 acre feet per acre were applied in two irrigations. The yield of five cuttings was 9.71 tons of hay per acre. In 1920, only one scanty irrigation was applied, because of a shortage of water, and only 2.5 tons of hay per acre were harvested.

In 1921, the east half of the area was plowed and prepared for orchard planting. The west half remained in alfalfa until the following year, when it was treated in the same way.

ARRANGEMENT OF PLANTINGS

The accompanying diagram (fig. 1) shows the arrangement of the first planting (block A). The second half (block B) duplicated the first except that the guard row of hardy pears was not repeated, but Hardy pears were used as pollinizers, and Satsuma plums were planted instead of Santa Rosa. The trees were planted twenty-seven and one-half feet apart each way.

The varieties planted were as follows: almond, Ne Plus Ultra and I.X.L.; peach, Lovell; apricot, Tilton; cherry, Chapman and Black Tartarian; Japanese plum, Santa Rosa and Beauty in Block A, Satsuma and Beauty in block B; apple, White Astrachan and Red Astrachan; prune, Robe de Sergeant and Agen (French); pear, Bartlett and (guard row) Hardy.

The rootstocks used were as follows: For Ne Plus Ultra almond, almond; for I.X.L. almond, peach; for peach, peach; for apricot, apricot; for cherry, Mazzard; for Japanese plum, Myrobalan; for apple, apple; for prune, Myrobalan; and for pear, Japanese pear (*Pyrus serotina*). In 1928, the almonds were removed because of the death of several trees and the badly diseased condition of several more, and replanted with pears on French root.

Pruning has been uniform and moderately light throughout the life of the planting. One irrigation a year, in addition to the rainfall, was sufficient to keep the trees growing thriftily until cover crops were planted. The plots were necessarily of three rows each. The scheme of planting of sorts requiring cross pollination was two of one variety and one of another (see fig. 1). This arrangement limited the plots to three rows in order to eliminate varietal differences between plots. If the guard row on the north be disregarded, this scheme gives seven three-row plots, duplicated in block *B*. The cultural treatments

	Check	Rye & Vetch	Melilotus	Check	North Mat Bean	Alfalfa	Check	
17	●	○	○	●	○	○	●	I. X.L. almonds
16	●	○	○	●	○	○	●	Ne Plus Ultra almonds
15	○	○	○	○	○	○	○	} Lovell peaches
14	○	○	○	○	○	○	○	} Tilton apricots
13	○	○	○	○	○	○	○	} Chapman cherries
12	○	○	○	○	○	○	○	} Black Tartarian cherries
11	●	○	○	●	○	○	●	Santa Rosa plums
10	●	○	○	●	○	○	●	} Beauty plums
9	●	○	○	●	○	○	●	White Astrachan apples
8	●	○	○	●	○	○	●	Red Astrachan apples
7	●	○	○	●	○	○	●	Robe de Sergeant prunes
6	●	○	○	●	○	○	●	} Agen prunes
5	●	○	○	●	○	○	●	} Bartlett pears
4	●	○	○	●	○	○	●	Hardy pears
3	○	○	○	○	○	○	○	
2	○	○	○	○	○	○	○	
1	○	○	○	○	○	○	○	
22	○	○	○	○	○	○	○	
21	○	○	○	○	○	○	○	
20	○	○	○	○	○	○	○	
19	○	○	○	○	○	○	○	
18	○	○	○	○	○	○	○	
17	○	○	○	○	○	○	○	
16	○	○	○	○	○	○	○	
15	○	○	○	○	○	○	○	
14	○	○	○	○	○	○	○	
13	○	○	○	○	○	○	○	
12	○	○	○	○	○	○	○	
11	○	○	○	○	○	○	○	
10	○	○	○	○	○	○	○	
9	○	○	○	○	○	○	○	
8	○	○	○	○	○	○	○	
7	○	○	○	○	○	○	○	
6	○	○	○	○	○	○	○	
5	○	○	○	○	○	○	○	
4	○	○	○	○	○	○	○	
3	○	○	○	○	○	○	○	
2	○	○	○	○	○	○	○	
1	○	○	○	○	○	○	○	

Fig. 1. Planting plan and arrangement of plots, block *A*. Block *B* duplicates block *A*, except as noted in the text.

included three plots in the nature of checks, which were given clean cultivation as ordinarily practiced in the Sacramento Valley: two plots with winter cover crops, one plot with summer crop, and one plot with a permanent cover crop of alfalfa. Another orchard served as a guard on the south. Starting at the north, the treatments of the seven plots were as follows: check (clean cultivation, with weed cover in winter); alfalfa; mat bean (*Phaseolus aconitifolius*) (a summer cover crop); check; *Melilotus indica*; rye and vetch; and check. The winter cover crops were planted September 22, 1924, for the first time, and in September of each year thereafter. The alfalfa was planted in the spring of 1925. The mat bean was first planted in May 1925, and in May of each succeeding year.

DATA OBTAINED FROM PLOTS

In order to determine the effect of these crops on tree growth and production, and to determine the way these effects are produced, observations of various sorts were made on the plots.

Records of the circumference of the trunk of each tree and of the yield of each tree have been kept. No differences have been seen in the behavior of the trees under these treatments up to the present time, except for a tendency for growth of the trunk to be slightly less in the apricots and peaches in block *B* in the alfalfa plot in 1927. Growth has been vigorous, and the early bearing sorts have given promise of good crops. The peach trees planted in block *B* averaged about twenty-five pounds of fruit per tree in 1927 and over fifty in 1928, while those in block *A* averaged nearly two hundred in 1927 and over two hundred in 1928. The apricot trees in block *B* averaged nearly fifty pounds per tree in 1927, though only twenty in 1928; while those in block *A* dropped from over one hundred in 1927 to forty in 1928.

Studies were made to determine seasonal changes in the soil solution, using the displacement method of Burd and Martin.⁽¹⁾ The only major modification made in their method was the use of the soil directly from the field at whatever moisture content it happened to have, rather than the adjustment of the moisture content to a standard percentage before displacement. It was thought that this modification would give a closer approximation to the relative proportion of the ions studied, than would a method which might dilute certain ions to a greater extent than others.

A series of samples was taken at weekly intervals from May to September 1926, in the north check plot of peaches in block *A*, in order to determine whether or not there were times during the summer when maxima or minima occurred which should be taken into account in further work. Two depths were studied: zero to three feet and three to six feet.

Considerable fluctuation was found in the concentration of all ions considered except hydrogen ion concentration, which was almost constant at pH 7.0 to 7.2 in the top three feet, and from 7.4 to 7.8 in the 3-6 foot samples; and phosphate concentration, which was almost without exception between the limits of 0.5 and 1.0 parts per million of total soluble phosphorus, expressed as PO_4 . There were, however, no marked maxima or minima between those dates. Sulfate varied

from 50 to 120 p.p.m., and nitrate from 130 to 500 p.p.m. of soil solution. The cations will be considered in a later paper. The differences existing between the top three feet and the second three feet were considered to be too small to warrant the extra labor involved in taking samples, so that all samples taken after 1926 are composites of the first four feet.

Field sampling was carried on with soil tubes of the type recommended by the Division of Irrigation. For all of the samples taken in 1927 and 1928, from twelve to eighteen four-foot samples were taken in the central area of a plot, and composited. Satisfactory duplicates could be obtained with this type of sampling.

In 1927, a series of plots was sampled once a month from March until August and thereafter at irregular intervals. Samples were taken from twenty-eight plots, which included all the peaches and the pears of both blocks. These two fruits were selected because they both do well under the climatic conditions found at Davis, and because they represented the stone and the pome fruits.

Specific resistance is given in tables 1 to 4. These data show a gradual drop throughout the summer, indicating an increase in the concentration of electrolytes. Less seasonal change occurs in the alfalfa plots than in the others. There seems to be some tendency for the resistance to be lower in the winter cover crop blocks, though this fact may have no significance. The moisture content of each sample is also given; no constant relation is apparent between resistance and moisture content.

The pH is fairly constant at about pH 7.0-7.6.

The data for phosphorus are not presented, for they followed the same level found in 1926. If one considers the values obtained by the colorimetric method from solutions which had not been evaporated and ignited, the level of inorganic PO_4 is much lower, being from about 0.5 parts per million to only a trace.

The data for nitrates in terms of parts per million of soil solution are presented in tables 5 to 8. There was a tendency to follow, in a general way, the trend of conductivity measurements, mentioned above. The drop in nitrates during the late winter period was also striking in most plots. The minimum concentration generally occurred about April in both 1927 and 1928, at which time specific resistance was highest. An interesting contrast is evident between peach series and pear series. In all six check plots in the pear series, the general level of nitrates was higher than that in the corresponding plot in the peach series. In the alfalfa plots, the differences were insignificant.

TABLE 1
SPECIFIC RESISTANCE OF DISPLACED SOLUTIONS IN OHMS AND MOISTURE CONTENT OF SAMPLES IN PER CENT OF DRY WEIGHT;
PEACH SERIES, BLOCK A

Date	Cheek		Alfalfa		Mat bean		Cheek		Milletotus		Rye-vetch		Check	
	Resist- ance	Water												
April 11, 1927.....	Ohms Per cent	24 690	Ohms Per cent	24 680	Ohms Per cent	23 400	Ohms Per cent	23 330	Ohms Per cent	23 380	Ohms Per cent	21 410	Ohms Per cent	24 500
May 9, 1927.....	21 380	21 400	20 270	20 300	22 300	19 360	20 380
June 13, 1927.....	23 270	23 270	20 270	20 300	22 290	22 290	20 270
July 11, 1927.....	18 210	18 230	17 230	17 270	16 180	14 180	15 260
August 17, 1927.....	23 360	23 320	15 230	14 270	22 270	20 320	21 370
October 5, 1927.....	13 230	13 270	15 16	13 270	15 250	12 250	15 230
December 2, 1927.....	18 270	18 270	16 290	15 340	18 230	17 230	18 270
January 19, 1928.....	18 150	18 230	22 22	19 230	19 270	22 200	19 230
April 24, 1928.....	21 270	18 270	18 320	18 380	21 320	19 250	18 420
May 21, 1928.....	17 320	22 320	16 22	19 270	17 200	17 240	17 250
June 18, 1928.....	16 290	16 240	17 13	17 280	20 220	16 13	15 360
July 9, 1928.....	13 240	13 230	12 13	11 230	12 220	13 190	14 240
August 8, 1928.....	13 230	13 230	12 18	11 220	12 210	10 160	12 190
September 15, 1928.....	17 200	17 350	17 17	17 220	17 160	18 150	14 190

TABLE 2
SPECIFIC RESISTANCE OF DISPLACED SOLUTIONS IN OHMS AND MOISTURE CONTENT OF SAMPLES IN PER CENT OF DRY WEIGHT;
PEACH SERIES, BLOCK B

Date	Check		Alfalfa		Mat bean		Check		Meliolotus		Kye-vetch		Check		
	Resist- ance Ohms	Water Per cent													
April 25, 1927.....	410	24	470	24	500	23	360	21	410	24	580	24	410	24	
May 23, 1927.....	300	17	340	13	230	21	300	16	370	15	210	14	370	16	
June 27, 1927.....	370	22	270	17	280	14	290	16	230	20	230	19	230	18	
July 25, 1927.....	190	16	310	23	160	12	270	14	210	16	170	15	240	15	
October 11, 1927.....	320	13	320	11	220	12	320	12	230	14	200	15	200	10	
January 16, 1928.....	310	22	320	21	180	18	260	20	200	22	180	22	200	20	
March 13, 1928.....	180	320	180	220	320	230	230	
April 12, 1928.....	320	21	330	20	360	21	450	19	360	21	250	19	450	19	
May 14, 1928.....	270	16	220	22	21	280	16	230	16	300	15	260	15	260	15
June 11, 1928.....	250	14	380	14	250	14	260	14	280	19	280	13	260	13	
July 12, 1928.....	340	15	410	15	230	12	230	17	250	12	200	14	320	13	
August 13, 1928.....	180	13	270	13	140	11	190	12	190	11	180	11	240	15	
September 5, 1928.....	240	12	500	15	200	12	230	14	200	13	180	12	180	10	

TABLE 3
SPECIFIC RESISTANCE OF DISPLACED SOLUTIONS IN OHMS AND MOISTURE CONTENT OF SAMPLES IN PER CENT OF DRY WEIGHT;
PEAR SERIES, BLOCK A

Date	Check		Alfalfa		Mat bean		Check		Mellotus		Rye-grass		Check	
	Resist- ance Ohms	Water Per cent												
April 18, 1927.....	350	24	500	24	500	22	510	21	360	21	350	22	500	21
May 16, 1927.....	320	21	330	19	330	21	340	19	270	17	330	18	400	23
June 20, 1927.....	270	22	380	24	360	19	300	22	290	21	250	18	340	24
July 18, 1927.....	250	19	310	15	250	20	250	17	190	17	140	14	290	20
September 19, 1927.....	270	18	410	14	270	15	200	17	230	22	260	17	410	13
October 24, 1927.....	180	18	230	13	170	13	150	14	200	17	150	17	160	18
January 11, 1928.....	180	20	140	22	180	21	180	23
February 13, 1928.....	200	24	320	22	270	23	230	24	320	23	340	22	230	22
April 10, 1928.....	360	410	410	410	360	320	320	270	310
May 2, 1928.....	300	21	17	270	23	320	22	190	240	450
May 23, 1928.....	270	20	270	17	180	210	270	300	16	240	19
June 20, 1928.....	240	22	320	17	270	18	270	19	260	18	240	20	230	20
July 11, 1928.....	200	20	360	15	230	20	280	17	230	18	230	15	250	18
August 10, 1928.....	210	17	17	230	13	180	13	180	13	210	13	190	12
September 19, 1928.....	17	270	15	180	16	14

TABLE 4
SPECIFIC RESISTANCE OF DISPLACED SOLUTIONS IN OHMS AND MOISTURE CONTENT OF SAMPLES IN PER CENT OF DRY WEIGHT;
PEAR SERIES, BLOCK B

Date	Check		Alfalfa		Mat bean		Check		Meliolus		Rye-grass		Check	
	Resist- ance Per cent	Water Per cent	Resist- ance Ohms	Water Per cent										
May 2, 1927.....	410	21	450	19	590	21	680	23	630	21	250	21	540	21
May 31, 1927.....	450	19	590	13	330	21	410	18	240	21	230	21	340	21
July 5, 1927.....	240	21	270	21	230	22	240	21	200	20	180	20	230	20
August 1, 1927.....	250	19	290	17	180	19	170	19	170	17	170	20	280	19
November 1, 1927.....	190	16	250	14	150	15	300	18	150	14	120	15	210	14
January 18, 1928.....	280	19	320	19	270	20	270	21	160	20	270	22	180	19
March 16, 1928.....	250	390	300	320	260	18	400	19	230	20
April 18, 1928.....	350	320	450	450	270	230	320
May 16, 1928.....	250	18	320	18	160	23	230	21	240	20	230	17	270	18
June 13, 1928.....	11	19	220	13	210	14	14	220	13
June 16, 1928.....	190	17	340	15	270	16	220	15	140	17	220	15	220	15
August 14, 1928.....	180	14	320	15	210	15	140	13	170	14	160	14	190	15
September 12, 1928.....	180	13	360	11	290	10	180	14	180	11	190	12	190	12

TABLE 5

NITRATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 10, 1927.....	117	157	157	109	176	101
April 11, 1927.....	101	78	112	67	135	140	78
May 9, 1927.....	73	176	111	157	140	211	148
June 13, 1927.....	185	127	149	132	121	254	168
July 11, 1927.....	288	104	157	199	317	322	314
August 17, 1927.....	75	125	200	225	150
October 5, 1927.....	193	175	88	156	142	293	160
December 2, 1927.....	136	434	360	322	322	341	347
January 18, 1928.....	353	75	136	174	124	155	244
March 20, 1928.....	200	34	47	87	45	58	77
April 24, 1928.....	145	67	102	62	100	110	93
May 21, 1928.....	113	65	113	120	122	157
June 18, 1928.....	145	100	163	165	93
July 9, 1928.....	162	100	106	139	180	125
August 8, 1928.....	165	84	142	230	131	195	187
September 15, 1928.....	205	82	175	195	269	324	271

TABLE 6

NITRATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 28, 1927.....	146	152	168	157	135	146	101
April 25, 1927.....	101	62	101	101	152	135	112
May 23, 1927.....	243	234	211	152	165	355	224
June 27, 1927.....	132	188	213	137	112	241	286
July 25, 1927.....	250	176	373	174	247	380	228
October 11, 1927.....	125	138	256	125	175	188	225
January 18, 1928.....	150	100	372	211	211	204	242
February 23, 1928.....	174	87	310
March 13, 1928.....	262	50	306	177	75	56	188
April 12, 1928.....	81	35	90	97	91	122	90
May 14, 1928.....	133	131	165	98	120
June 11, 1928.....	206	111	245	206	160	162	157
July 12, 1928.....	177	55	222	210	200	104	181
August 12, 1928.....	225	146	250	215	200	219	229
September 5, 1928.....	205	82	175	195	269	324	271

TABLE 7

NITRATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 21, 1927.....	236	146	162	130	121	225	247
April 18, 1927.....	112	180	185	146	258	202	118
May 16, 1927.....	161	110	213	198	198	190	189
June 20, 1927.....	280	130	211	243	217	285	204
July 18, 1927.....	274	173	263	242	496	606	244
September 19, 1927.....	238	75	160	240	288	400	156
October 24, 1927.....	262	81	256	431	312	427	500
January 11, 1928.....	372		347	250		174	211
February 13, 1928.....	329	87	236	409	124	124	360
April 10, 1928.....	221	43	61	130	93	92	215
April 26, 1928.....	142	37	200	83			
May 2, 1928.....	168		202	166	315	150	125
May 23, 1928.....	218	93	397	285	225	137	345
June 20, 1928.....	283	263	250	237	217	237	325
July 11, 1928.....	356	87	288	225	238	375	278
August 10, 1928.....	300		224	387	311	268	387
September 19, 1928.....		89		394			516

TABLE 8

NITRATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
April 4, 1927.....	123	112	152	202	225	191	169
May 2, 1927.....	202	73	135	101	112	270	123
May 31, 1927.....	230	238	173	173	306	587	248
July 5, 1927.....	328	223	308	299	326	418	370
August 1, 1927.....	305	150	487	238	547	490	306
November 1, 1927.....	387	121	485	325	385	435	304
January 18, 1928.....	223	98	223	273	422	236	422
March 18, 1928.....	172	40	190	171	177	107	287
April 18, 1928.....	140	46	75	101	138	160	178
May 16, 1928.....	261			267	280	250	261
June 13, 1928.....				382	377		391
July 16, 1928.....	410	95	150	359	620	331	363
August 14, 1928.....	437	131	330	562	418	434	525
September 12, 1928.....	594	79	262	600	600	462	500

In the mat bean plots the differences were small, but both the plots of the pear series were higher than the corresponding plots of the peach series. The differences in the winter cover crop plots were even more pronounced than those in the check plots. This difference between species may be explained, possibly, by the fact that the growth and yield of the peach trees was much greater than that of the pear trees. Another plausible explanation is the fact that peaches are considered by the grower to be "heavy nitrogen feeders." The plots of the peach series were less variable than the pears, all of the plots tending to change in the same direction and to show smaller differences between the highest and lowest ones.

The alfalfa plots had a tendency to be low in nitrates, especially under the pear trees. In the peach series the alfalfa plots are the lowest in over half of the samples; while in the plots of the pear series they are lowest on twenty-two out of twenty six dates. This phenomenon is more striking in 1928 than in 1927.

In the mat bean plots, the general behavior was similar to that of the checks.

The Melilotus plots showed a high degree of variability. Only in the pears of Block *B* is there a marked increase in nitrates over the check plots in 1927, the other three series having shown, in general, little tendency to rise above the checks. The curves in 1928 closely approximate those of the checks.

The rye and vetch plots had the highest general level of nitrates through most of the year in 1927. After about November they fell very rapidly, to a low point in the spring. In 1928, the curves were close to those of the checks, often falling below them, as may be seen from inspection of tables 5 to 8. Figure 2 shows the average of the nitrate concentration of the north and center checks from tables 5 to 8. The seasonal variation and difference between peach and pear plots is illustrated. Figure 3 shows the data for alfalfa plots from the same tables. The two sharp maxima are probably due to local nitrate accumulations.

The data for nitrates, expressed in terms of parts per million of dry soil, are presented in tables 9 to 12. These data give an approximation to the concentrations that might be expected from "one to one" extracts. They indicate that the seasonal changes mentioned above are not the result of dilution or concentration due to changes in moisture content of the soil alone.

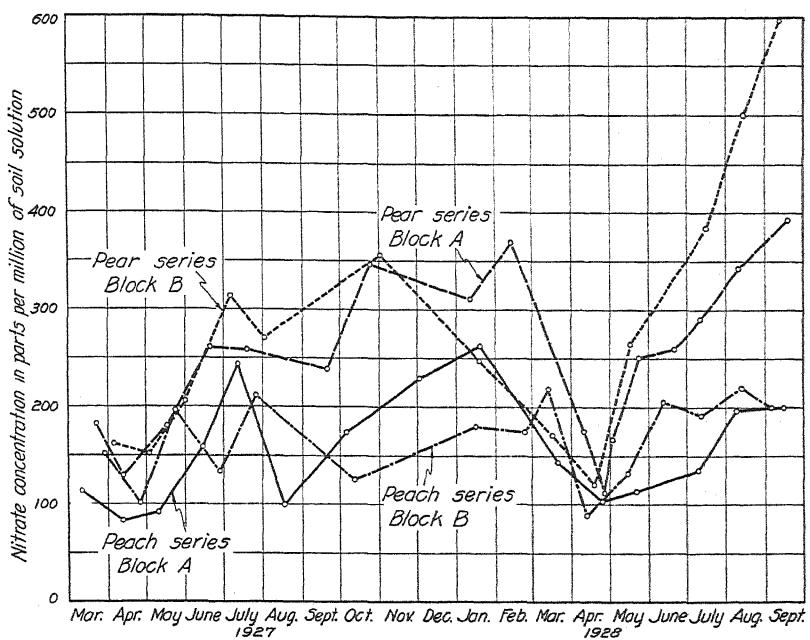


Fig. 2. Average nitrate content of north and center check plots, in parts per million of displaced solution.

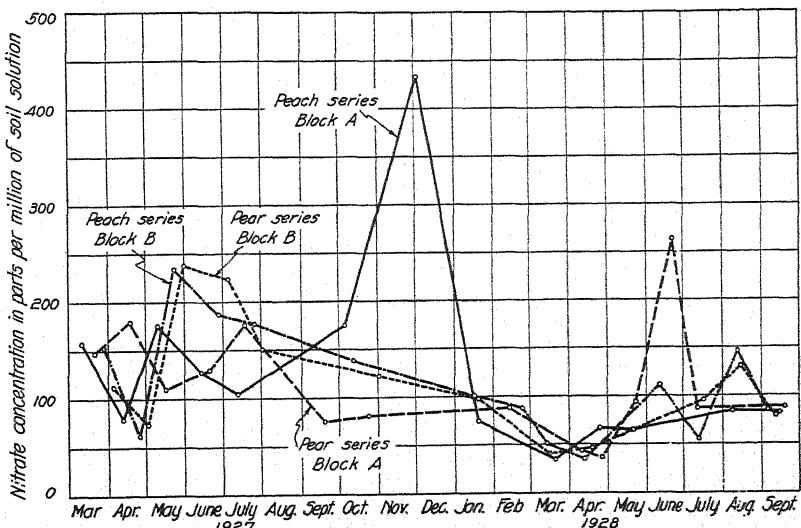


Fig. 3. Nitrate content of alfalfa plots, in parts per million of displaced solution.

TABLE 9

NITRATE CONTENT OF DISPLACED SOIL SOLUTION IN PEACH SERIES, BLOCK A,
IN PARTS PER MILLION OF DRY SOIL
(Calculated from table 5)

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 10, 1927.....	29	39	39	27	44	47	25
April 11, 1927.....	24	21	26	16	28	31	19
May 9, 1927.....	15	37	22	33	27	43	33
June 13, 1927.....	43	25	29	29	27	69	35
July 11, 1927.....	52	19	27	30	44	48	50
August 17, 1927.....	17			18	40	47	23
October 6, 1927.....	25	26	11	23	17	44	21
December 2, 1927.....	24	69	54	58	55	61	66
January 19, 1928.....	64	12	20	31	23	28	46
March 20, 1928.....	42	7	10	18	9	12	15
April 24, 1928.....	30	12	18	7	19	20	19
May 21, 1928.....	19	12		18	23	20	27
June 18, 1928.....	23		17		26	25	16
July 9, 1928.....	21		16	12	18	22	16
August 8, 1928.....	21	11	16	28	13	23	24
September 14, 1928.....	35	15	19	33	48	45	38

TABLE 10

NITRATE CONTENT OF DISPLACED SOIL SOLUTION IN PEACH SERIES, BLOCK B,
IN PARTS PER MILLION OF DRY SOIL
(Calculated from table 6)

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 28, 1927.....	37	38	42	39	34	38	25
April 25, 1927.....	23	11	20	18	31	27	22
May 23, 1927.....	41	30	44	24	25	50	38
June 27, 1927.....	28	30	47	27	22	48	52
July 25, 1927.....	40	40	45	24	40	57	34
October 11, 1927.....	16	15	31	15	25	28	23
January 16, 1928.....	33	21	67	42		45	48
February 23, 1928.....	38	20	66				
March 13, 1928.....	52	10	60	35	14	11	38
April 12, 1928.....	17	7	19	18	19	23	17
May 14, 1928.....	21			21	26	15	18
June 11, 1928.....	29	15	34	28	31	21	20
July 12, 1928.....	27	8	27	36	24	15	24
August 12, 1928.....	29	19	28	26	23	24	34
September 5, 1928.....	23	11	45	36	36	27	35

TABLE 11

NITRATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A, IN PARTS PER MILLION OF DRY SOIL
(Calculated from table 7)

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 21, 1927.....	59	37	41	33	30	56	62
April 18, 1927.....	27	43	41	31	54	44	25
May 16, 1927.....	34	21	45	38	34	34	44
June 20, 1927.....	62	31	40	52	46	51	49
July 18, 1927.....	52	26	26	64	84	85	49
September 19, 1927.....	43	11	24	41	63	68	20
October 24, 1927.....	47	11	33	60	53	60	90
January 11, 1928.....	74	73	58	36	46
February 13, 1928.....	79	19	54	98	29	27	79
April 10, 1928 (estimated)*.....	44	8	12	26	19	18	43
April 26, 1928.....	6	42	17
May 2, 1928.....	35	46	36	63	30	25
May 23, 1928.....	43	16	83	57	45	22	66
June 20, 1928.....	62	43	45	45	39	47	64
July 11, 1928.....	71	13	58	38	42	55	50
August 10, 1928.....	51	29	50	40	35	46
September 19, 1928.....	13	63	72

* This set of figures calculated on an estimated moisture content of 20 per cent.

TABLE 12

NITRATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B, IN PARTS PER MILLION OF DRY SOIL
(Calculated from table 8)

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
April 4, 1927.....	31	28	38	51	56	48	42
May 2, 1927.....	42	14	28	23	23	57	26
May 31, 1927.....	45	31	36	31	58	100	46
July 5, 1927.....	69	46	68	63	64	84	74
August 1, 1927.....	58	26	90	45	93	98	57
November 1, 1927.....	62	17	70	59	54	65	43
January 18, 1928.....	42	19	45	57	84	62	80
March 16, 1928.....	34	8	38	34	34	21	57
April 18, 1928 (estimated)*.....	28	9	15	20	27	32	34
May 16, 1928.....	47	56	56	43	47
June 13, 1928.....	50	53	51
July 16, 1928.....	70	14	24	54	105	50	54
August 14, 1928.....	61	20	50	72	59	59	79
September 12, 1928.....	77	9	26	84	66	55	60

* This set of figures calculated on an estimated moisture content of 20 per cent.

Among the most striking differences thus far observed were those in the sulfate content, given in tables 13 to 16, between the peach series and the pear series. In the great majority of cases throughout this period, the sulfate content of the solution from the peach series was higher than that of the corresponding pear plot. The differences were greater and more consistent than were those of the nitrates pointed

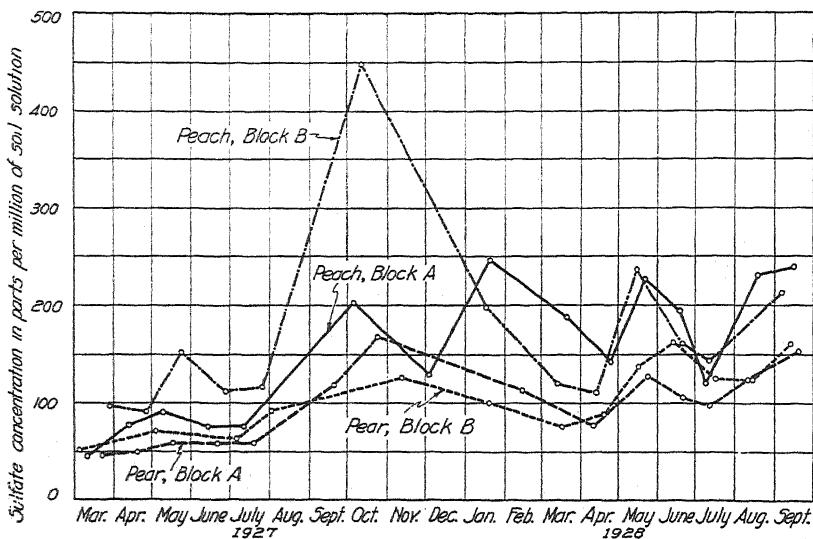


Fig. 4. Average sulfate content of the three check plots, in parts per million of displaced solution.

out above. The variability of the peach series with regard to sulfate was likewise greater than that of the pear plots. Differences between treatments were not consistent enough to be considered significant. It should be noted that the tendency of the maxima to appear in the fall and the minima in spring corresponded to the tendencies noted for nitrate, though by no means in so clear a manner. The data expressed on the dry weight basis showed the same features. The average of the three check plots is shown graphically in figure 4.

TABLE 13

SULFATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK A, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 10, 1927.....	79	160	54	21	46	42	38
April 11, 1927.....	52	73	92	45	102	104	135
May 9, 1927.....	81	104	102	83	120	75	108
June 13, 1927.....	85	99	64	113	121	75	29
July 11, 1927.....	83	143	124	62	156	89	82
October 5, 1927.....		250	228	202	194		
December 2, 1927.....	130	164			146	92	127
January 19, 1928.....	317		189		193	125	174
March 30, 1928.....	320	135	168	92	205	155	151
April 24, 1928.....	215	169	147	119	89	142	91
May 21, 1928.....	284	153		231	258	182	227
June 18, 1928.....	247		109	149	93	142	187
July 9, 1928.....	162		98	169	185	226	179
August 8, 1928.....	250		203	210	295		
September 15, 1928.....	310	191	197	201	193	238	237

TABLE 14

SULFATE CONTENT OF SOIL SOLUTION IN PEACH SERIES, BLOCK B, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 28, 1927.....	92	78	39	52		31	146
April 25, 1927.....	120	146	104	104	80	115	49
May 23, 1927.....	146	193	177	196	68	68	113
June 27, 1927.....	61	125	63	159	97	144	114
July 25, 1927.....	196	86	188	109	79	128	42
October 11, 1927.....	332	169	372	652		283	362
January 16, 1928.....	178	160	207	201	83	226	216
February 23, 1928.....	103	193	164				
March 13, 1928.....	185	114	188	115	107	182	209
April 12, 1928.....	187	141	127	76	102	135	68
May 14, 1928.....	240			231	235	233	238
June 11, 1928.....	190	139	110	168	198	67	121
July 12, 1928.....	144	64	146	154	170	101	131
August 12, 1928.....	229		190				
September 5, 1928.....	203	81	193	225	211	277	211

TABLE 15

SULFATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK A, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 21, 1927.....	75	29	22	29	22	15	38
April 18, 1927.....	66	14	34	45	26	41	38
May 16, 1927.....	40	47	57	65	60	38	71
June 20, 1927.....	63	63	40	56	40	38	59
July 18, 1927.....	46	68	62	89	43	65	41
September 19, 1927.....	105	89	101	131	103	81
October 24, 1927.....	168	354	205	351
February 13, 1928.....	117	91	94	119	123	87	103
April 10, 1928.....	58	120	84	84	151	102	88
May 23, 1928.....	102	134	113	153	120
June 20, 1928.....	102	69	100	128	98	109
July 11, 1928.....	106	103	116	90	94	106	96
August 10, 1928.....	125	128	109	100	97	138
September 19, 1928.....	219	152

TABLE 16

SULFATE CONTENT OF SOIL SOLUTION IN PEAR SERIES, BLOCK B, IN PARTS PER MILLION OF DISPLACED SOLUTION

Date	Check	Alfalfa	Mat bean	Check	Meli-lotus	Rye-vetch	Check
March 4, 1927.....	42	20	27	52	46	62	60
May 2, 1927.....	85	32	25	62	26	92	67
July 5, 1927.....	58	51	59	40	51	52	95
August 1, 1927.....	123	106	92	92	114	63	59
November 11, 1927.....	193	86	59	175
January 18, 1928.....	98	82	81	133	75	121
March 16, 1928.....	83	66	82	70	97	76
April 18, 1928.....	77	55	76	86	103	141	98
May 16, 1928.....	140	168	118	138	101
May 13, 1928.....	155	105	167
July 16, 1928.....	158	89	95	125	138	111	88
August 14, 1928.....	113	123
September 12, 1928.....	179	115	102	152	107	160	150

DISCUSSION AND CONCLUSIONS

As a result of these studies, which are in the nature of a progress report, it is possible to point out certain points of difference between the changes induced in the soil solution by trees and by cereals. Burd and Martin⁽²⁾ showed a marked drop in nitrate content at the end of the growing season for cereals. The data presented here do not show this for trees under Davis conditions, but show a tendency for a rise to occur during the growing season. It might be thought that the cover crops, whether planted or natural, as in the check plots, show the same tendency as do cereals. The alfalfa plots, however, have their minima at the same time as do the winter cover crop plots, although the growing season of alfalfa corresponds more nearly to that of the trees. The mat bean plots, which are nearly bare in winter, show a similar drop in the spring. It appears that, with the exception of alfalfa, nitrification exceeds utilization throughout the summer months. This is in fairly good agreement with the data of Lyon, Heinicke and Wilson,⁽⁴⁾ who find nitrates to be at their maximum in June to August and to decrease in October. They report no data for the winter months. The minimum in the spring may be due to withdrawal by roots, which are growing at that period.

Burd⁽³⁾ has pointed out that soils depleted in chloride and nitrate ions are high in sulfates. A similar phenomenon is seen in the comparison of the peach and pear series, the plots of the peach series being higher in sulfates and lower in nitrates than the plots of the pear series. The coefficient of correlation between the concentrations of these two ions is, however, very low, and the seasonal fluctuations of the two ions do not show a reciprocal relationship. It is possible that the bicarbonate relationship may offer at least a partial explanation of these discrepancies, though adequate data on this point are not available at the present time. The fact that nitrates are generally somewhat higher and sulfates lower than in most of the cropped soils reported on by Burd and Martin suggests that with more intensive cropping the differences between species noted here might be more striking, and certain anomalies in the figures might disappear.

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DAILY AND SEASONAL AIR AND SOIL TEMPERATURES AT DAVIS, CALIFORNIA*

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INTRODUCTION

Since 1924 certain phases of soil temperature have been studied at Davis, California, in order to ascertain the influence of various soil conditions, of moisture, and of the shading effect of crops upon soil temperatures.

The data herein reported will show that the ranges in thermal environment of various parts of a growing plant-top, stems, and roots are apparently very great. For example, the leaves and branches on July 17, 1925, were in atmosphere heated to 116° Fahrenheit; the stem (unshaded) just below the ground surface was in soil having a temperature of 143°; while the roots would be in a medium at 107° for the 3-inch depth, and 84° for the 24-inch depth.

Bacteria and fungi of many varieties may live at low temperatures in the soil. The limit below which most cultivated plants are practically inactive lies in general between 40 and 45° F. Bacterial activity which increases the supply of available nitrogen is stimulated by relatively high temperatures while at extremely high temperatures this activity is reduced to a degree as unimportant as when too low temperatures prevail. Thus a knowledge of the range of

* The cooperation of several individuals is hereby acknowledged: Mrs. J. B. Simmons, Messrs. J. W. Tidmore and Fred Flint for their assistance in collecting the data; and Mr. J. L. Lynde for preparing the final copy of the figures.

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temperature at various depths in the soil, will be helpful in the interpretation of bacterial activity.

The effect of soil temperatures on the chemical reactions taking place in soils is shown by the varying character of the soil solution throughout the year. The diffusion of the dissolved material away from the soil and through the roots and other tissues of the plant are probably hastened with a rise of temperature.

The sharp contrast between the highly decomposed soils of wet tropical regions and the moderately decomposed soils of the polar regions is due to the greater rainfall in the tropics and the influence of high temperatures in promoting rapid chemical action in the soil.

LOCATION OF EXPERIMENTAL SITE

The site under study lies on a recent alluvial fan, the apex of which is approximately 14 miles west of the site and the base is about 7 miles east. The slope in the immediate vicinity ranges from 5 to 10 feet per mile in a general southeast direction. The nearest hills are those at the apex of the fan. The soil which is a member of the Yolo series is derived mainly from sedimentary rocks and is brown in color when moist.

The texture of the surface soil is loam and at a depth of 3 feet it changes to a fine sandy loam of a light brown color. From well borings made in the immediate vicinity it is found that no hardpan or bedrock is encountered within 150 feet of the surface. The surface water table is normally at a depth of about 20 feet while the depth of the irrigation wells are approximately 120 feet. The source of the soil moisture in the upper six feet of soil was from rainfall alone as it has been determined⁽¹⁰⁾ that with the Yolo loam "water tables at ten feet or more below the surface would be below the maximum height of capillary rise and would result in no movement of water to the surface."

As the rains occur during the winter only and the air temperatures are lower during the rainy season the moisture content in the surface soil is naturally higher from September to May than during the rest of the year. During the dry season the moisture content of the surface soil was decreased by direct evaporation from the bare plots, as the land was not cropped, and all weeds and other vegetation was kept down by regular cultivation. No crops or vegetation have been allowed to grow on this plot after 1923.

SOIL AND ATMOSPHERIC CLIMATE

The "soil climate" is to a large extent dependent on the "atmospheric climate," as many investigators have shown that the changes in barometric pressure, temperature, humidity, and other climatic factors influence the soil climate. Some of the recent contributors to this phase of research have been Bouyoucos and McCool,⁽¹⁾ Taylor,⁽¹⁴⁾ Walker,⁽¹⁰⁾ Harrington,⁽³⁾ Shaw,⁽⁹⁾ and Smith.⁽¹¹⁾ Certain elements of soil climate, as for instance temperature, have pronounced effects on plant growth, diseases, bacterial activity, etc. These problems have received widespread attention during the past few years as by Jones, Johnson and Dickson,⁽⁴⁾ Camp and Walker,⁽²⁾ Valleau, Kenny and Kinney,⁽¹⁵⁾ Mason,⁽⁷⁾ and many others. Russell⁽⁸⁾ has made an areal analysis of California climates and suggested a classification of the various types based on the classification scheme of Vladimir Köppen.⁽⁵⁾ Russell characterizes the climate of the Sacramento Valley where this soil temperature work has been carried on as "Hot Summer Mediterranean, olive climate, with its warmest month averaging above 71° F. The type of Mesothermal climate characterized by at least three times as much rainfall in its wettest winter month as received during its driest month is commonly known as Mediterranean."

In the latest annual summary of the United States Department of Agriculture Weather Bureau⁽⁶⁾ the average annual precipitation for Davis is given as 17.03 inches with the month of January having an average of 3.87 inches and the driest months, June and July having an average of 0.01 inch. The warmest month, July, at Davis averages 74.6° F.

SOIL MOISTURE VARIATIONS

Determinations of the moisture content of the area where these soil temperature studies were carried on were made at frequent intervals. The samples were obtained by means of a soil tube, at the following depths, in inches: 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60, and 60-72 in four locations in the area. The samples were dried to constant weight at 212° F and the percentage calculated on oven dry basis.

With subsoil drainage unrestricted, which condition existed in this area, during the winter season the soil retained moisture up to its normal moisture capacity or to its field moisture capacity of 20-22

per cent in the loam surface soil (0-36 inches) and 16 to 18 per cent in the fine sandy loam subsoil (36-72 inches). This condition was reached after the seasonal rainfall totaled about 3 inches which appeared sufficient to replenish the moisture lost during the previous dry season. Many moisture equivalent determinations made on part of the soil samples from which the soil moisture tests were made show that the moisture equivalent of the surface loam soil is around 20 per cent and of the fine sandy loam subsoil is 16, or approximately the amount of moisture normally present in the soil during the rainy season.

During the dry season of the year, on account of the fact that this area was not irrigated, the moisture content of the soil decreased. The surface 4 inches naturally dried out to the greatest extent so that by September, when the rains usually start, it contained approximately 5.00 per cent. The moisture content of the next layer (4-8 inches), dropped to about 12 per cent and for the section, 8-12 inches, to about 15 per cent. In the sections, 12-24 inches, and 24-36 inches, the lowest moisture content was about 18 per cent. In the various sections of 36-72 inch depth the soil was a fine sandy loam and the lowest moisture content was around 15 per cent. With no crop growing, and cultivating the surface 4 inches once a month, there was a changing moisture content extending to a depth of 72 inches. The loss of moisture from the soil was brought about mainly by vapor movement, possibly aided by some capillary movement. In another portion of this area, experiments were conducted with various types of paper mulches which will be reported in the future. Certain phases of this study have been reported⁽¹²⁾ but mention is made here in order to offer evidence about soil moisture movements. By covering the soil with an unperforated black paper at the beginning of the dry season it was found that the surface 4-inch section contained approximately 20 per cent of moisture at the end of the dry season. The 4-8 inch section was drier and its moisture content at the end of the dry season was generally about 12 per cent. Beads of moisture collected on the underside of the paper and as only the 0-4 inch area maintained its moisture content and the area immediately below it was much drier it is clear that moisture movement in the vapor phase is an important consideration.

INSTALLATION OF RESISTANCE THERMOMETERS

Electrical resistance thermometers were standardized and placed at the depths of $\frac{1}{2}$, 3, 6, 12, 24, and 36 inches in the bare cultivated area. They were put in place by digging a small hole, carefully preserving the soil from the various depths. The thermometer bulbs were then inserted in the undisturbed soil horizontally so that they were at least 8 inches from the wall of the excavation. The soil was then replaced, care being exercised to attain the same structure as originally existed at the various depths.[‡] The resistance thermometers were connected to an overhead cable which extends for a distance of 150 feet to a small frame structure wherein an automatic Leeds and Northrup temperature recorder was installed. The temperature from each individual resistance thermometer was recorded every 15 minutes, day and night, in degrees Farhenheit. Air temperatures herein recorded were obtained at a height of four and one-half feet above the soil at the Davis Station of the United States Weather Bureau located approximately 1400 feet northwest of the soil temperature plots. A shelter house similar to the United States Weather Bureau type is located on the area where the soil temperature studies were made, and tests made at different seasons were found to be in close agreement with those occurring at the Davis Weather Bureau Station. Therefore, the data for the curves were taken from the records of the latter. Temperatures of the immediate soil surface are not reported herein although occasional records were obtained by use of the copper bulb thermometer.⁽¹¹⁾ The soil temperature ranges, occurrence of maximums and minimums with respect to sunrise and sunset, and the diurnal range for the various depths during the warmest week in 1925 have been previously reported.⁽¹²⁾ From the data given in the accompanying figures similar information can be obtained for any part of the periods shown. In preparing the figures it was necessary to record the data by hourly intervals but care was always exercised that the actual maximums and minimums were accurately shown.

[‡] Temperatures are now being obtained in another phase of the project, at depths of 48, 60, and 72 inches also. The results will be reported in a later publication.

INTERPRETATION OF FIGURES

The prevailing sky condition for each particular day is given, being indicated as cloudy if the sky was overcast the greater part of the 24-hour period although there may have been a few hours with a clear sky as allowed in the Weather Bureau classification. The same is shown relative to direction of the wind and in case it was variable it is so indicated. During the winter months the north winds are cold while during the dry summer months they are hot. The southwest wind is cool in the summer and warm in the winter as it comes through the Golden Gate over the San Francisco Bay and then swings northward over land areas for a distance of about 30 miles before it reaches this area.

The rainfall occurring on any particular date is also shown so that the effect of this climatic element can also be noted.

The data herein reported is for two continuous periods, February to October, 1925, and December, 1926 to July, 1927. Occasionally the instrument was stopped because of making adjustments, failure of current, or for repairs; such periods are indicated by breaks in the curves. After installing a direct current motor, trickle charger and 24-volt battery having 50 ampere hours capacity, there have been relatively few interruptions in operation. Since this change was made the recorder has been operating on almost perfect schedule and in a period of 24 hours the usual time error is not over 2 minutes. Corrections are made when necessary and this error is not cumulative. When operating on the alternating current the line load varied and the current would be cut off for short periods making the time error greater. The temperatures shown on the graphs are within one degree of accuracy and the time error is less than 5 minutes.

TEMPERATURE RECORDS FOR 1925 PERIOD

The first set of figures (1-31 inclusive) covers the interval from February 23, 1925 to September 28, 1925 and shows the temperature ranges that crops would be subjected to when planted early in the spring and harvested before the heavy rains commenced in the fall. In this period of 31 weeks (217 days) a large number of crops are sown and harvested in California. For emphasis, it is again stated that the area where these studies were made was not irrigated.

Air Temperature Ranges During 1925 Period.—The minimum air temperature of 30° was reached at 6 A.M. on March 10, 1925 (fig. 3) while the sky was clear and at the beginning of a six-day period without rain and with a prevailing wind direction from the north. The maximum air temperature of 116° was reached at 1 P.M. on July 17 (fig. 21) on a clear day with north wind. In the autumn the minimum air temperature before September 28 was nearly 45° in the week of September 21 (fig. 31). The range in air temperature during the 1925 period was 86°.

Soil Temperatures at One-half Inch Depth for 1925 Period.—The minimum soil temperature for soil thermometer No. 1 (one half inch depth) occurred at practically the same time as the minimum air temperature on March 10 (fig. 3) and was 29° F. The maximum soil temperature at one-half inch depth which was of sufficient duration to affect the deeper soil amounted to 143° and occurred on July 17 (fig. 21) at 2 P.M., one hour after the maximum air temperature. A higher maximum of 146° (fig. 23) was reached at this depth but this was not reflected in the temperatures of the deeper soil layers because the soil was drier and the duration was not as long on July 17. The minimum temperature in the autumn before September 28 for the one-half inch depth was 55° and occurred at practically the same time as the minimum air temperature (fig. 31). There was a range in temperature during the 1925 period at a depth of one-half inch in the soil of 114° or 28 degrees greater than in the range in the air temperatures during the same period.

Soil Temperatures at 3-inch Depth for 1925 Period.—The minimum soil temperature for the 3-inch depth (thermometer No. 2) was 42° and occurred at 7 A.M. on March 10 (fig. 3) one hour after the minimum air temperature had been reached. The maximum temperature at this depth amounting to 107° was reached on July 17 (fig. 21), two hours after the maximum air temperature. The lowest temperature for the 3-inch depth after the summer months and before September 28 was 68° and occurred during the last week. The range in temperature for the 3-inch depth was 65° or 21° less than that of the air and 49° less or only 57 per cent of the range for the one-half inch depth.

Soil Temperatures at 6-inch Depth for 1925 Period.—The soil temperatures obtained at a depth of 6 inches are indicated by curve No. 3 on the figures. The minimum temperature at this depth was 44° and occurred at 8 A.M. on March 10 (fig. 3) two hours after the minimum air temperature. The maximum temperature of 101° was reached on

July 17 (fig. 21) which gives a range of temperature at the 6-inch depth of 57° during this period. This range was 88 per cent of the range which had occurred at the 3-inch depth or 50 per cent of the range for the one-half inch depth. After the summer period the minimum temperature reached at the 6-inch depth was 73° which occurred during the week of September 21 (fig. 31). At the depth of 6 inches during this period of 31 weeks the soil temperature did not fall below 44° F.

Soil Temperatures at 12-inch Depth for 1925 Period.—Daily changes in soil temperatures are clearly evident at the 12-inch depth shown in figures 1-31 inclusive and as has been previously reported.⁽⁸⁾ The temperatures obtained for this depth are indicated on the figures by curve 4. The minimum temperature was 48° at 12 noon on March 10 (fig. 3) or six hours after the minimum air temperature. The maximum temperature of 93° was reached at 1 A.M. on July 18 (fig. 21) which was twelve hours after the maximum air temperature. A range in temperature of 45° is shown for the 12-inch depth or 79 per cent as great as occurred at the 6-inch depth, or 40 per cent of the range for the one-half inch depth. The minimum temperature at the end of the period was 73°, the same as for the 6-inch depth.

Soil Temperatures at 24-inch Depth for 1925 Period.—The temperatures for the 24-inch depth are shown as curve 5 on the figures. The minimum was 52° and occurred on March 12 (fig. 3) at midnight or sixty-six hours after the minimum soil temperature. The maximum of 87° was reached at 12 noon on July 19 which was twenty-three hours after the maximum air temperature. The range of 35° for this depth was 78 per cent as great as had occurred at the 12-inch depth or nearly 31 per cent of the range for the one-half inch depth. The temperatures during the last week (fig. 31) at this depth were fairly constant, dropping to 77° during the last two days. In general there are no regular increases accompanied by decreases in temperature during a twenty-four hour period at this depth, there being either a gradual warming or a gradual cooling.

Soil Temperatures at 36-inch Depth for 1925 Period.—The minimum temperature for the 36-inch depth (curve 6) was 53° which occurred at midnight on March 12 (fig. 3) at the same time as the minimum for the 24-inch depth. The maximum of 84° occurred at 11 A.M. (fig. 22) on July 22 which was 118 hours after the maximum air temperature. The range in temperature for this depth was 31° and was 90 per cent of that at the 24-inch depth or 27 per cent as great as had occurred in the one-half-inch section. Throughout the

entire last week (fig. 31) the temperature for this depth was uniform. It will be noted that curve 6 which indicates the temperature changes for this depth is smoother than the others.

Effect of Character of Sky, Rain, and Wind During 1925 Period.—It is difficult to show the effect of the rain, wind, or cloudiness by the scale used in the accompanying figures. Average conditions for each twenty-four hour period only are shown. The velocity of the wind naturally varies considerably in a twenty-four hour period. It was regularly noticed that sudden changes in wind direction, velocity or cloudiness were reflected in the soil temperatures to a depth of at least 3 inches when these conditions endured for as short a period as ten minutes. Preceding the middle of May in general the north wind has a cooling effect on the soil temperatures while later the opposite is true. The south winds are opposite in effect to the north winds. Many times during the summer months the wind is from the north during the daylight hours and at night there is a slight southerly cooling wind. This could be clearly shown only by using such a scale for the figures whereby the velocity and direction of the winds could be indicated at least by fifteen-minute intervals. The effect of "day breezes" during the summer months is therefore warming as the air moves a long distance over land areas while the "night breezes" are cooling because they come from over water surfaces and only a short distance over land areas.

Clear weather before the middle of May is accompanied by increasing soil temperatures but when combined with north winds a decreasing effect is noted. Cloudy weather without rain culminated by southerly winds aids in reducing the daily range in soil temperature for the surface 24 inches of soil. Particular periods can be observed when the effect of cloudiness or wind direction are more noticeable than at other times. The effect of rainfall on soil temperatures is most effectively shown in figures 5 and 6 where during the period of March 29-31, inclusive, there was a total rainfall of about 3 inches. This rain came after a period of five dry weeks.

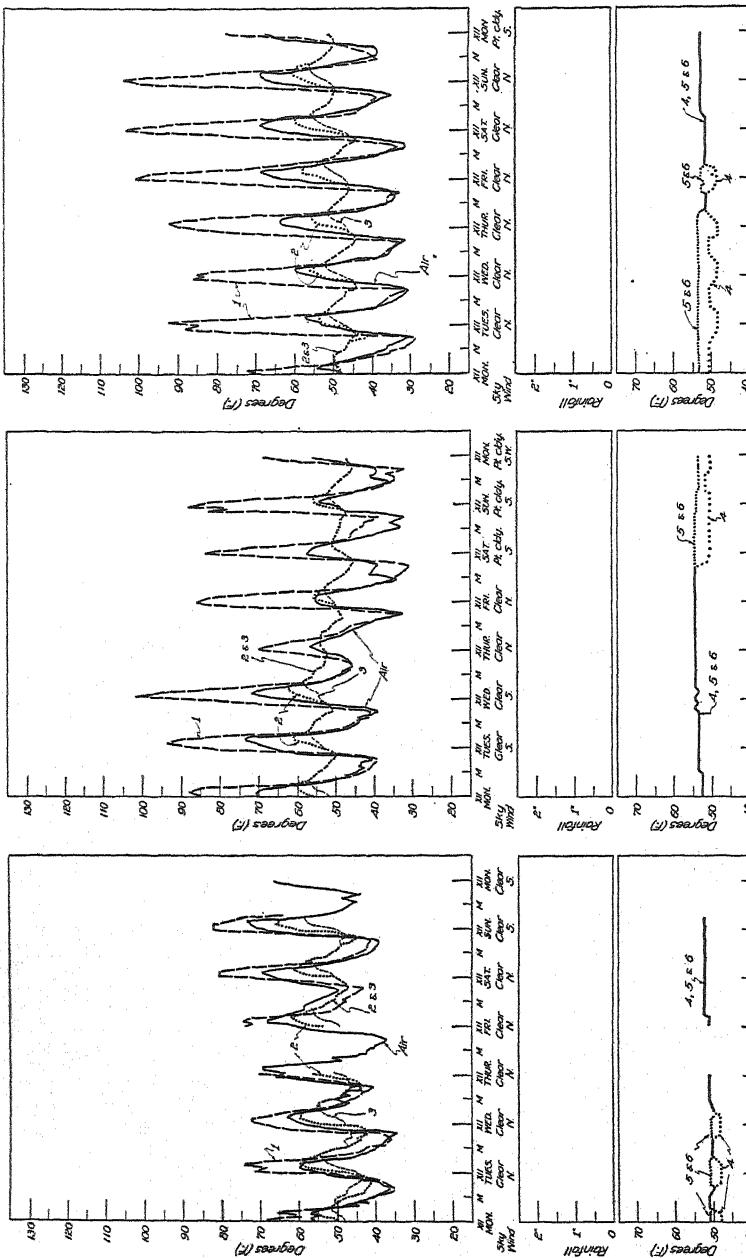


Fig. 1.—Week of February 23, 1925.

Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 2.—Week of March 2, 1925.

Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

Fig. 3.—Week of March 9, 1925.

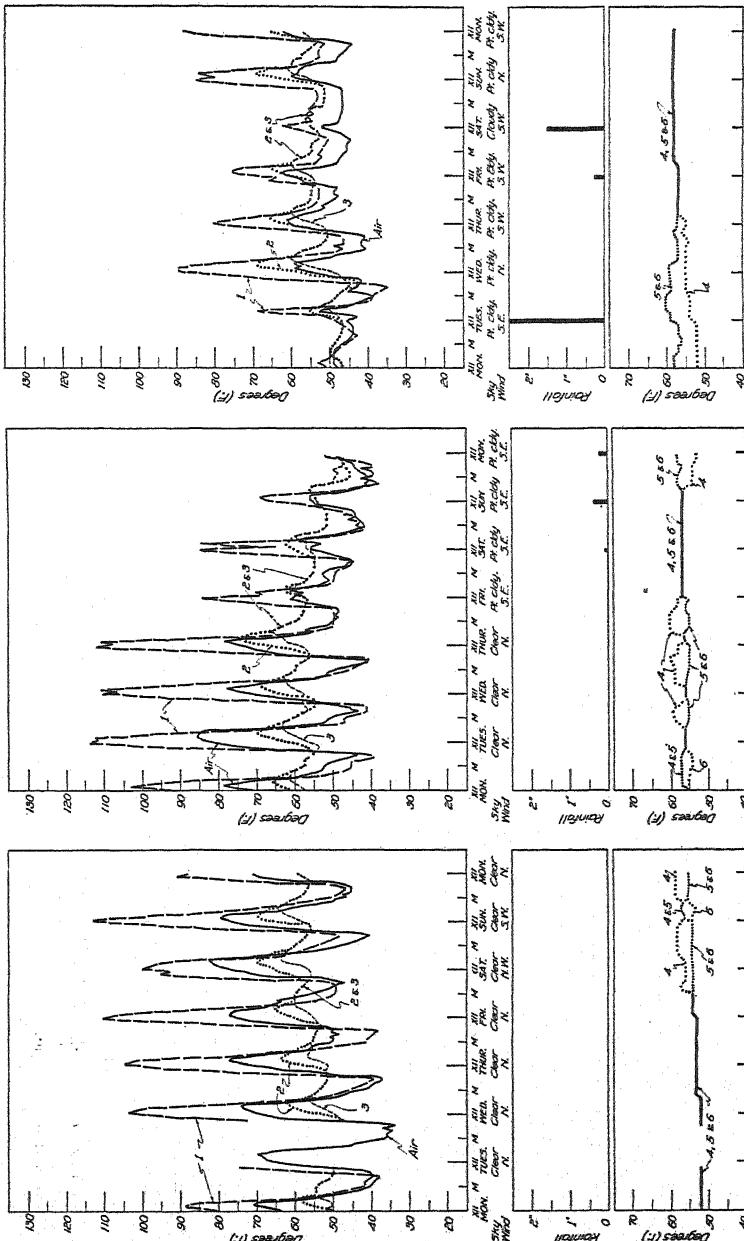


Fig. 4.—Week of March 16, 1925.
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

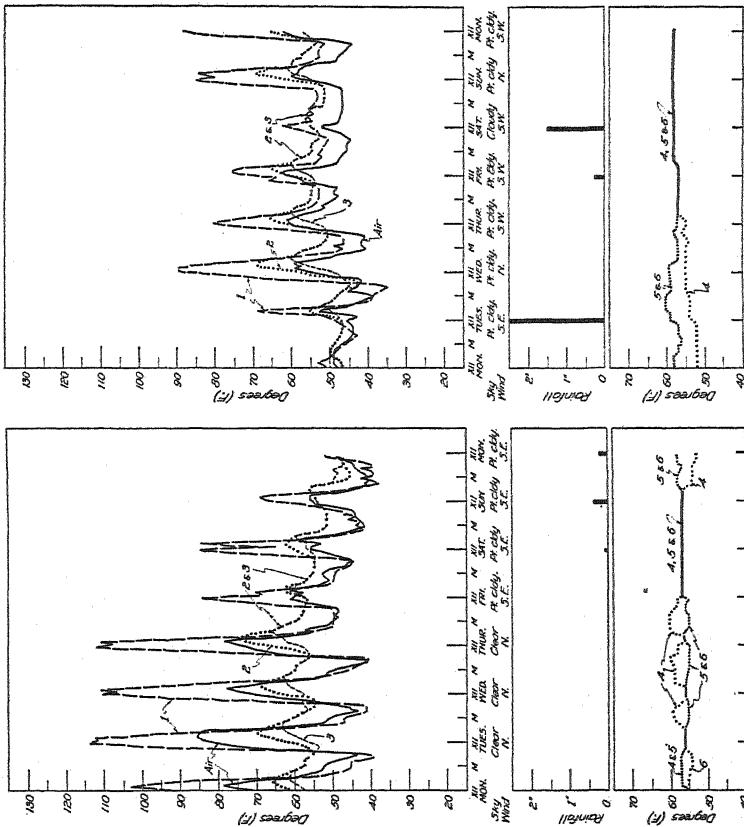


Fig. 5.—Week of March 23, 1925.
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

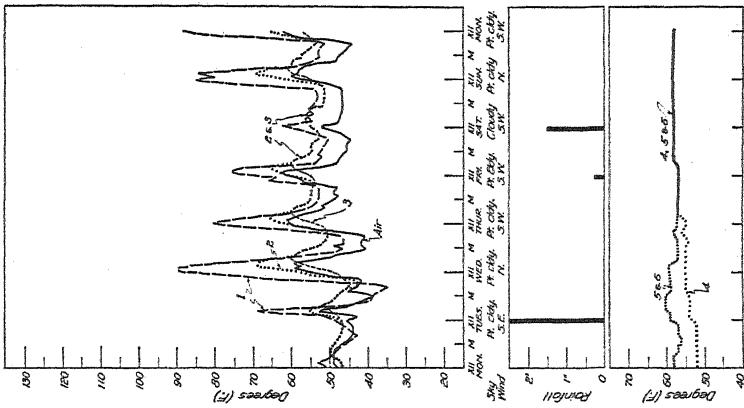


Fig. 6.—Week of March 30, 1925.
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

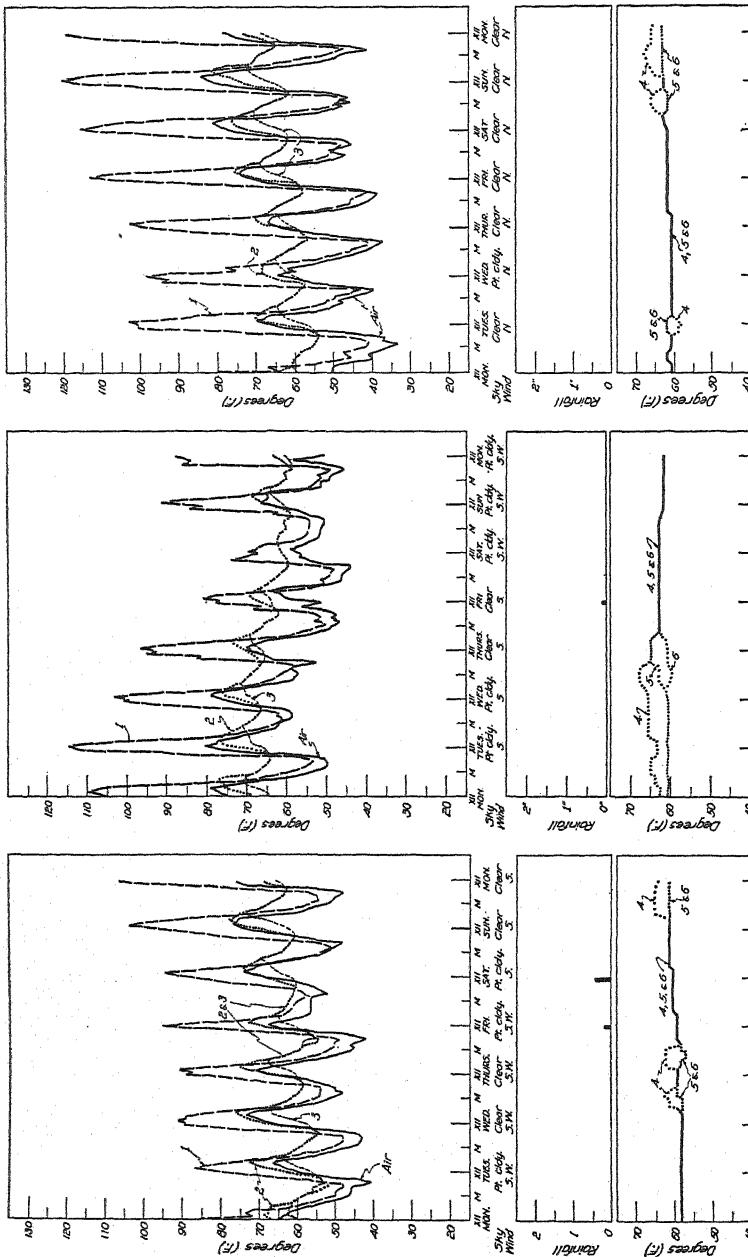


Fig. 7.—Week of April 6, 1925.
Soil and air temperatures and atmos-

Fig. 8. Week of April 13, 1925.

Fig. 9.—Week of April 20, 1925.

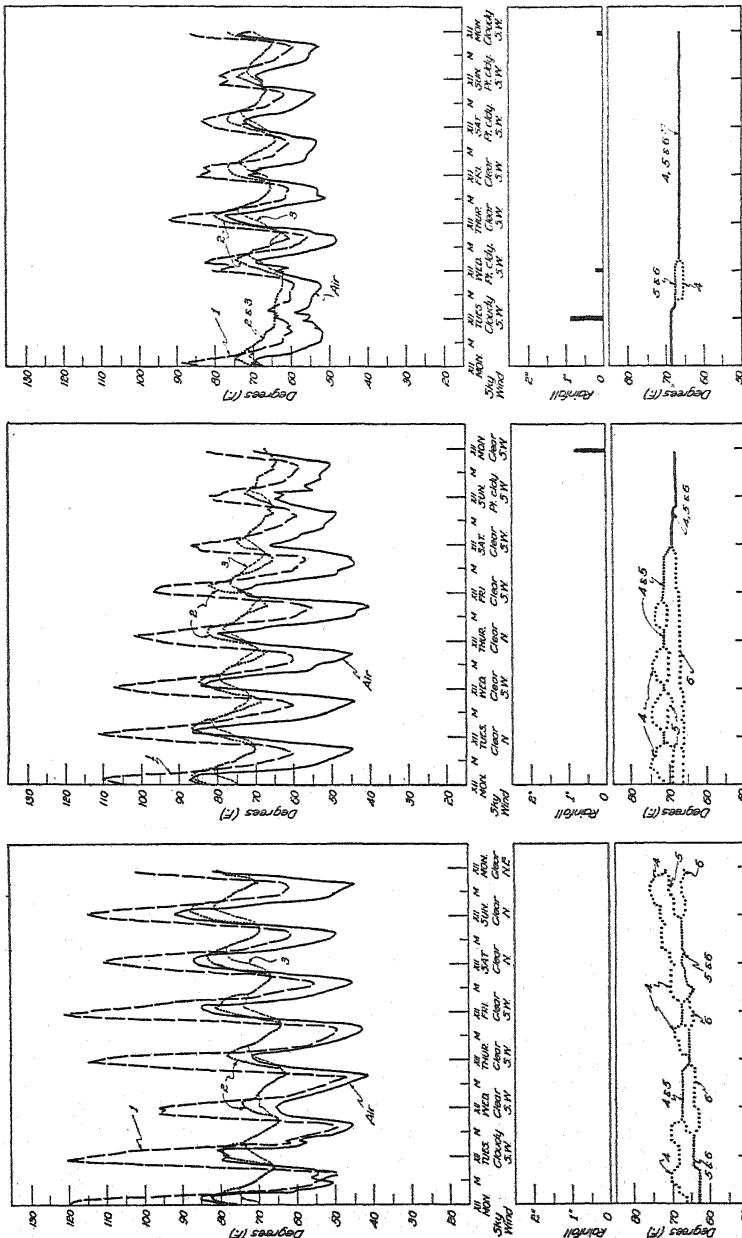


Fig. 10. Week of April 27, 1925

Soil and air temperatures and atmosph
g a

Fig. 11. Week of May 4, 1925

atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

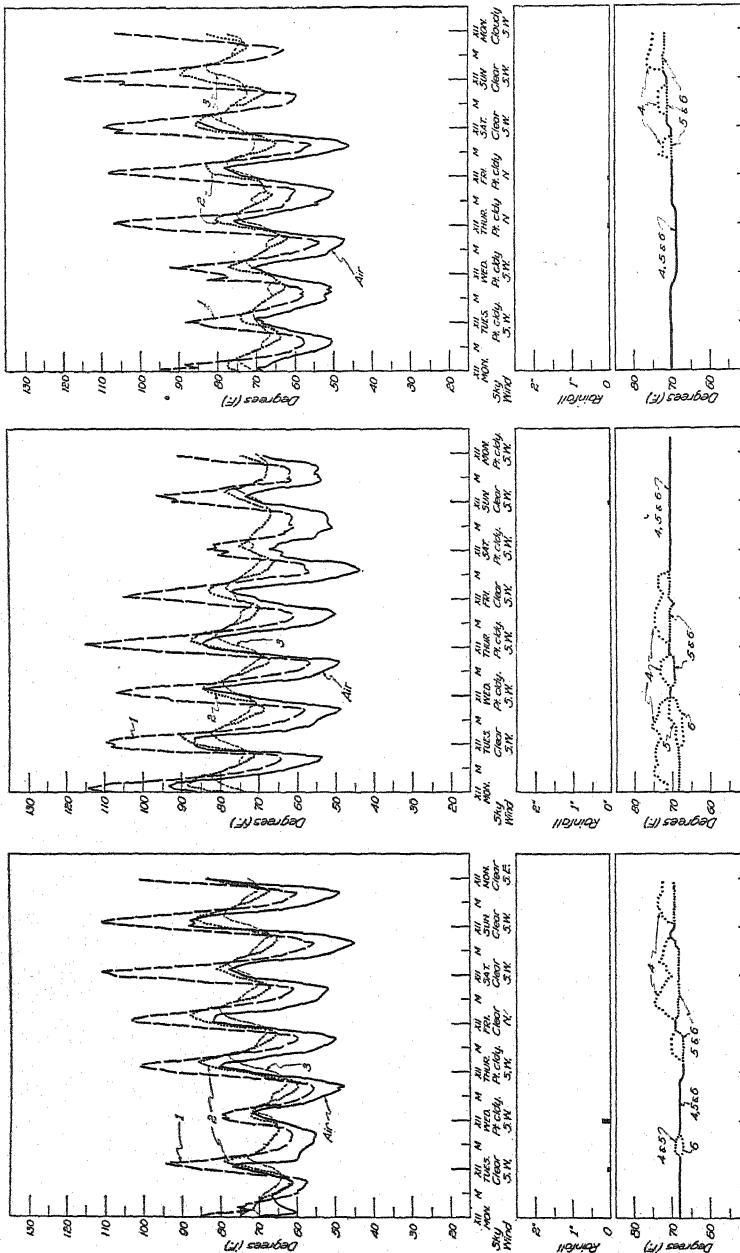


Fig. 13. Week of May 18, 1925
 Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 14. Week of May 25, 1925
 Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 15.—Week of June 1, 1925.

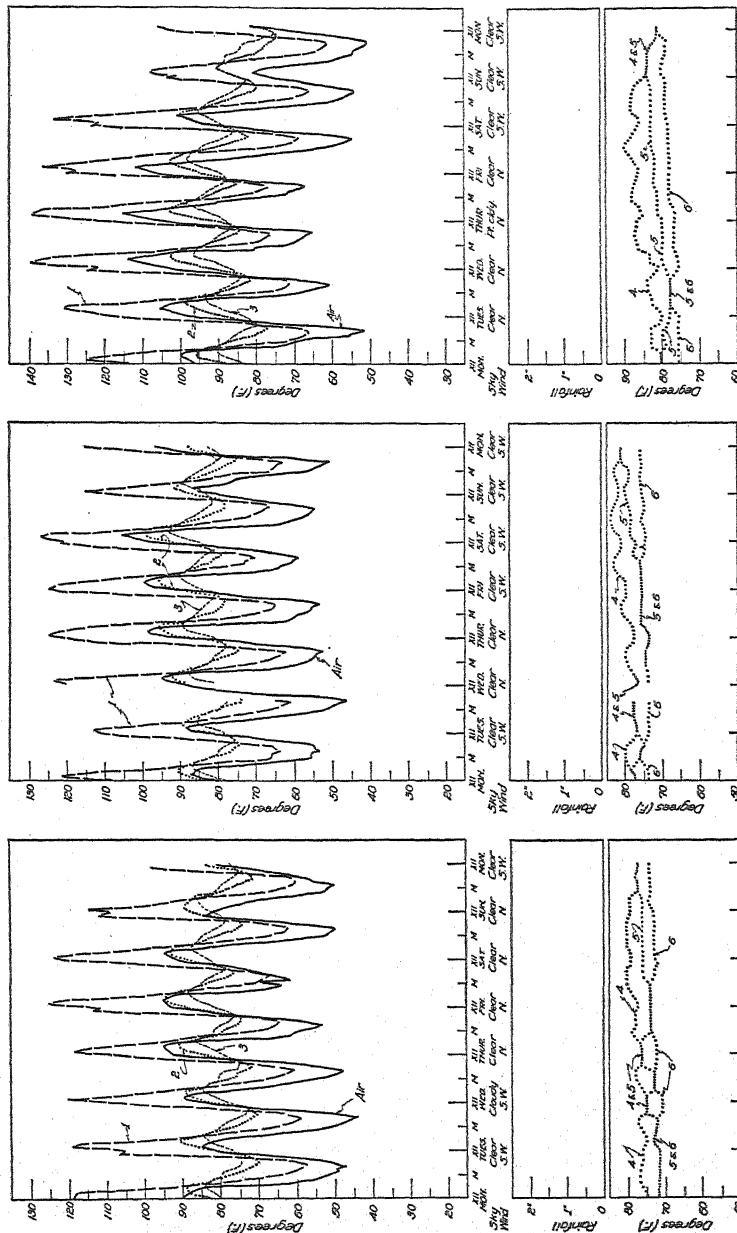


Fig. 16. Week of June 8, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 17. Week of June 15, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

Fig. 18.—Week of June 22, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

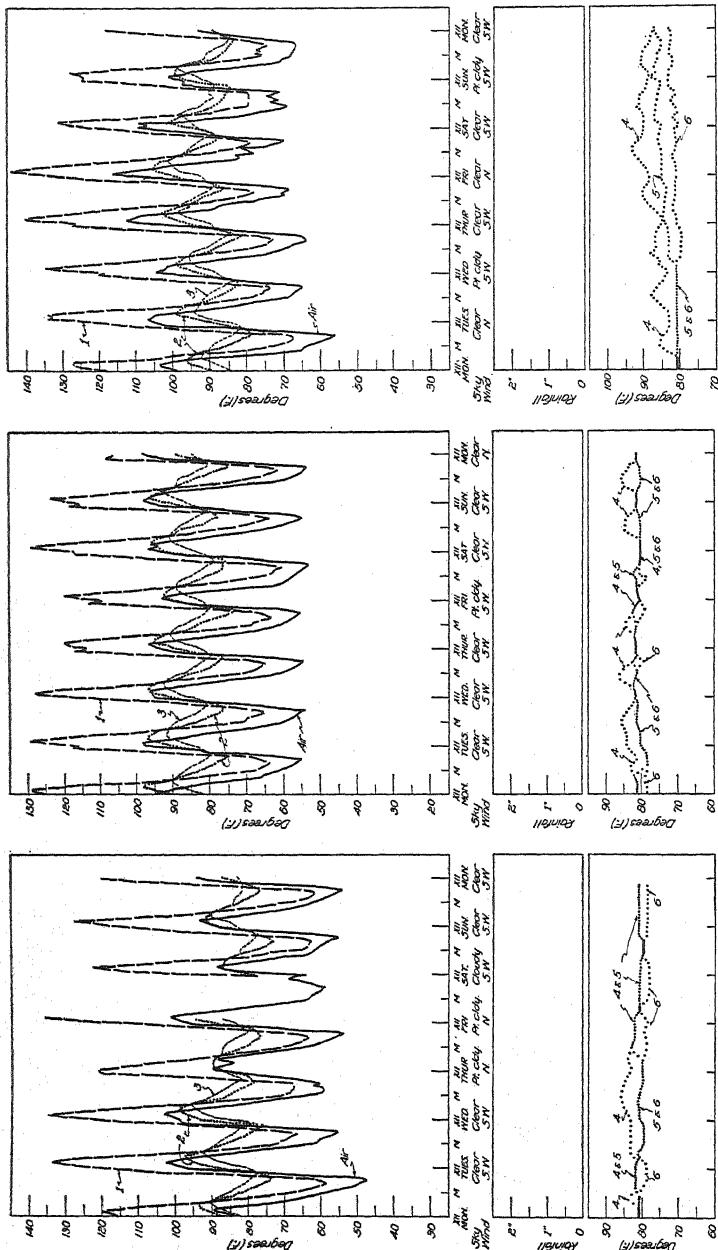


Fig. 19. Week of June 29, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 20. Week of July 6, 1925
Fig. 21.—Week of July 13, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

Fig. 21.—Week of July 13, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

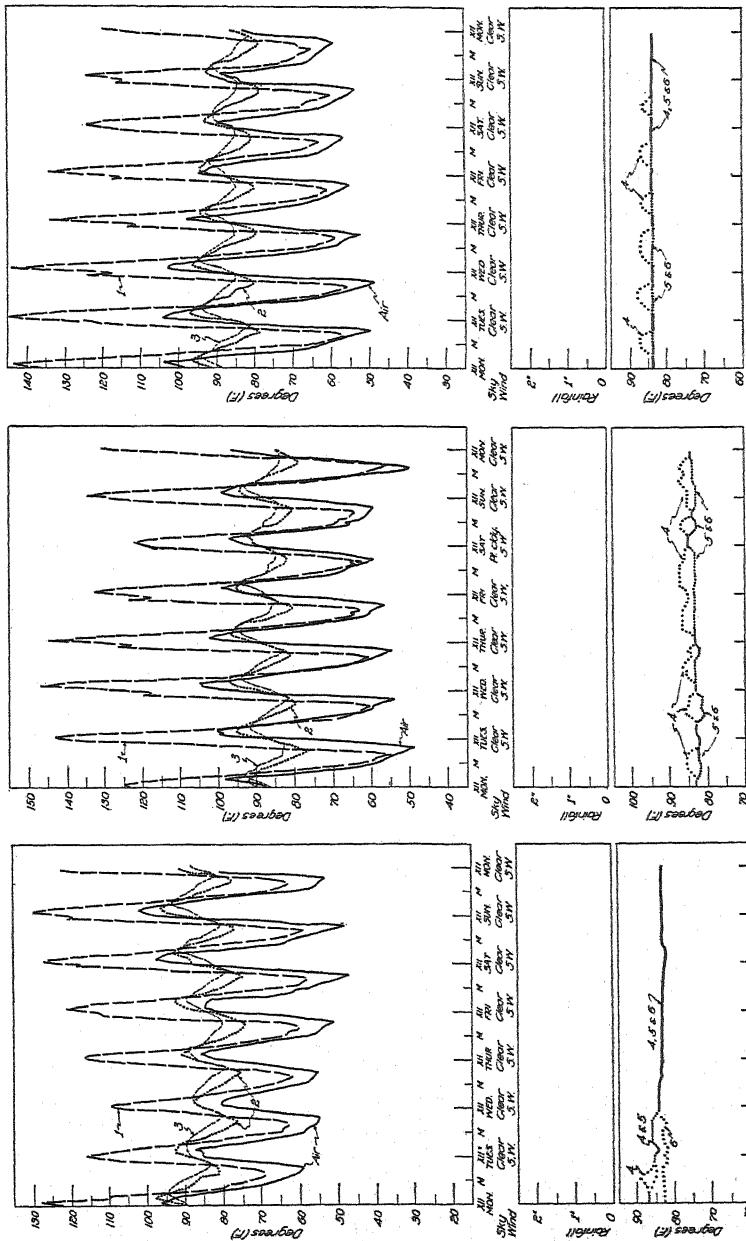


Fig. 22. Week of July 20, 1925
Soil and air temperatures and atmos-

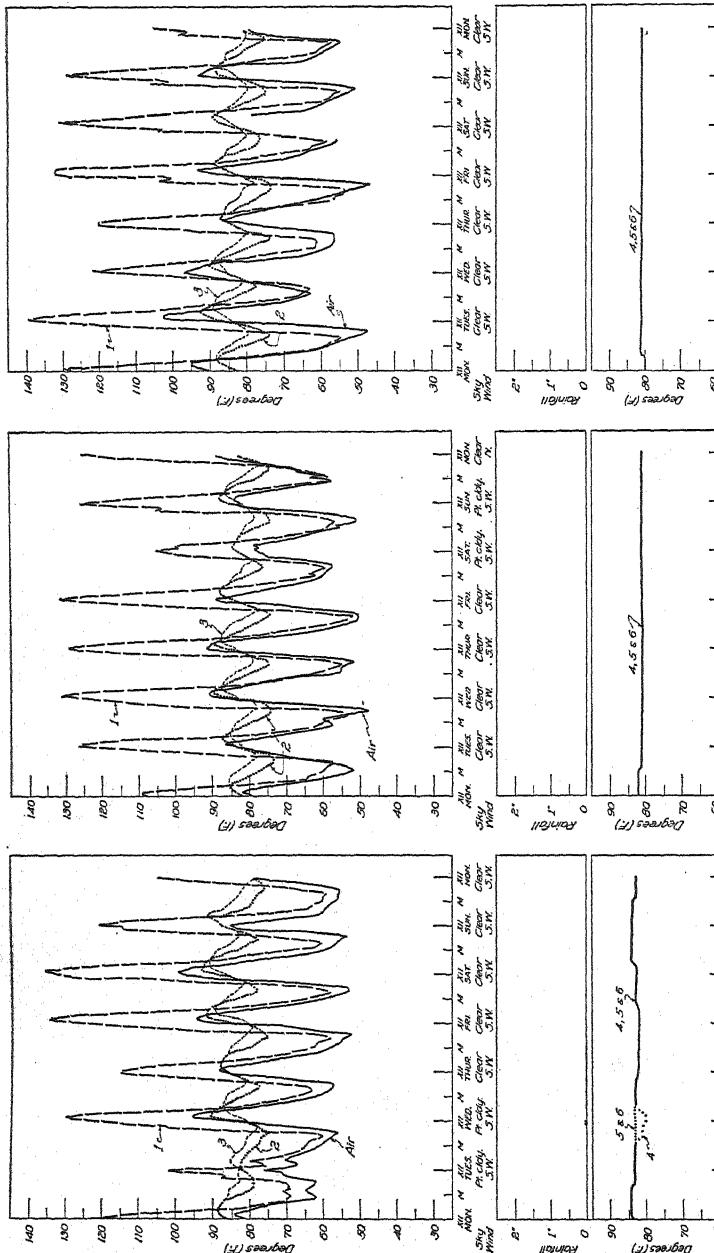


Fig. 25. Week of August 10, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 26. Week of August 17, 1925
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 27.—Week of August 24, 1925.

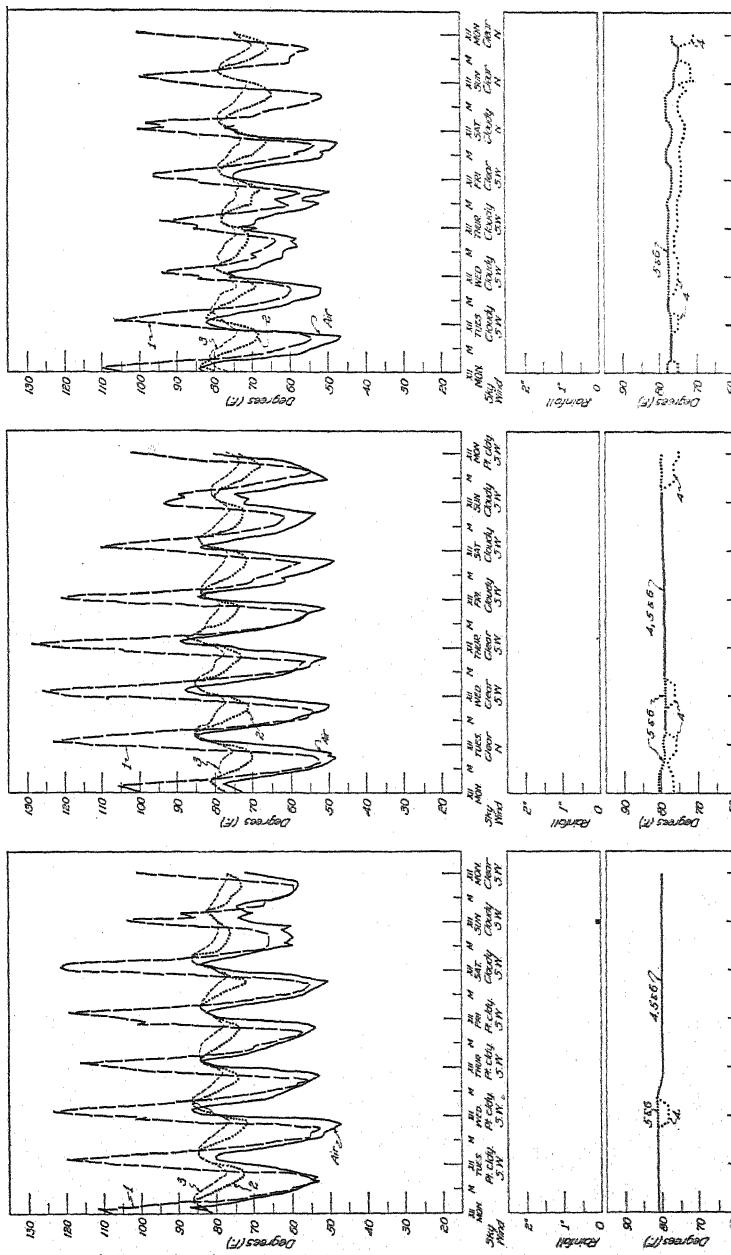


Fig. 28. Week of August 31, 1925

TEMPERATURE RECORDS FOR 1927 PERIOD

The next set of figures (32-57) inclusive takes into consideration the temperature conditions that fall sown crops would be subjected to. This period extends from December 20, 1926 to June 20, 1927 and is also of aid in comparing certain similar dates with the first set (figures 1-31). The lowest air temperatures at Davis usually occur during December or January and frequently the highest occur about the middle of June so this set of data might prove of value from that viewpoint as well as for fall sown crops and short season spring crops.

Air Temperature Ranges During 1927 Period.—The lowest air temperature was 24° and occurred at 5 A.M. on December 27 (fig. 32) while the sky was clear and the air calm. This was preceded by a period of four days when the prevailing wind direction was from the north. For the five days from December 27 (fig. 32) to December 31 (fig. 33), inclusive, the minimum ranged from 24° to 26° F. The maximum air temperature of 105° occurred at 2 P.M. on June 15 (fig. 57) during a period of clear weather when the prevailing wind was from the north. During the periods when the maximum and minimum air temperatures were reached, the north wind had either a cooling or warming effect depending on whether the land area over which it passed was cold as in winter or warm as in summer. The range in the air temperatures during the 1927 period of observation was 81°.

Soil Temperatures at One-half Inch Depth for 1927 Period.—The minimum soil temperature at the one-half inch depth (curve 1) was 32° and occurred at 5 A.M. on December 27 (fig. 32) at practically the same time when the minimum air temperature occurred. The surface soil at this time was moist. The maximum temperature of 108° was reached at 3 P.M. on June 15 (fig. 57), one hour after the maximum air temperature had occurred and when the surface soil was dry. A range of 76° is indicated in the temperatures at the one-half inch depth which is about 94 per cent as great as that of the air temperatures. It is particularly of interest to note that although the temperature of the air four and one-half feet above the soil reached a minimum of 24° the lowest temperature of the soil at a depth of one-half inch was 32°—or 8° higher.

Soil Temperatures at 3-inch Depth for 1927 Period.—The temperatures at the 3-inch depth are shown in the graphs as curve 2. A minimum of 37° occurred at 7 A.M. on December 27 (fig. 32) two

hours after the minimum air temperature, although a slightly lower temperature is shown on December 24 with a higher air minimum. The maximum of 96° was reached at 4 P.M. on June 15 (fig. 57) two hours after the air maximum. A range of 59° occurred at this depth from December 20 to June 20. This was slightly over 77 per cent as great as had occurred at the one-half inch depth during the same period. The lag in the soil temperatures as compared to the air temperatures is clearly shown at the time of the occurrence of the maximum and minimum for this soil depth.

Soil Temperatures at 6-inch Depth for 1927 Period.—The minimum temperature of 41° was reached at the 6-inch depth (curve 3) at 10 A.M. on December 27 (fig. 32) five hours after the minimum air temperature, while the maximum of 91° occurred at 4 P.M. on June 15 (fig. 57) or two hours after the maximum air temperature. The range in temperature during this period, December 20 to June 20, at the 6-inch depth was 50° or about 85 per cent as great as had occurred at the 3-inch depth in the same interval or 66 per cent of the range for the one-half inch depth.

Soil Temperature Changes at 12-inch Depth for 1927 Period.—The temperatures obtained at the 12-inch depth are indicated by curve 4 which shows a minimum of 42° on December 26 (fig. 32). The same temperature was also reached on December 27 (fig. 33). The maximum temperature for this depth was 84° at 10 P.M. on June 15 (fig. 57), eight hours after the maximum air temperature was reached. The range in temperature for this depth was 42° or approximately 55 per cent as great as had occurred at the one-half inch depth during the same interval. The figures, 32-57, clearly show as do the preceding ones that there is in general a distinct rise and fall in the soil temperatures at the 12-inch depth.

Soil Temperature Changes at 24-inch Depth for 1927 Period.—The minimum temperature recorded for the 24-inch depth (curve 5) was 48° and occurred at 11 A.M. on December 31 (fig. 33). This time was 102 hours after the minimum air temperature of 24° had been reached. The maximum of 79° at 10 A.M. on June 17 (fig. 57) was forty-four hours after the maximum air temperature of 105°. The range in temperature for this depth was 31° between the winter and June 20, or mid-summer. This range was only 41 per cent as great as had occurred at the one-half inch depth during the same period. In general, as previously shown, there is no marked rise and fall in the daily soil temperatures in this area at a depth of 24 inches, this characteristic being confined to the surface 12 inches.

Soil Temperature Changes at 36-inch Depth for 1927 Period.—The temperatures recorded for the 36-inch depth are shown as curve 6 in the figures. The minimum for this depth was 52°, occurring at 12 noon on December 31 (fig. 33) or 103 hours after the minimum air temperature of 24° had occurred. The maximum of 76° was reached on June 17 (fig. 57) or six hours after the maximum for the 24-inch depth. The range in temperature was 24° or slightly over 31 per cent as great as had occurred in the one-half inch section during the same period, December 20 to June 20.

The lag in temperatures between the 24-inch depth and the 36-inch depth are clearly indicated in the accompanying figures.

Effect of Character of Sky, Rain, and Wind During 1927 Period.—From December 20 to April 18 there was some rainfall every week with the exception of the week beginning March 21 (fig. 45). During this moist period the air temperature curves and the soil temperature curves 1, 2, and 3 which represented the one-half inch, 3-inch, and 6-inch depths and all of which are in the upper portion of the figures (figs. 32-57), are very close together. There was no great rise and fall in temperatures such as was shown during the same part of the 1925 period. The fact that the surface soil was moist for this length of time (December-April) combined with the large amount of cloudiness reduced the temperature ranges in the surface soil. Later in the summer of 1927 there was little rain, and the surface soil being drier, there was a noted change in the degree of spread of curves 1, 2, 3, and the air temperature curve. The week of February 14 (fig. 40) is outstanding for the similarity of the curves in the upper portion of the figure. Maximum temperatures for that week occurred on Friday when the sky was clear with a southwest wind. The week of May 23 (fig. 54) was clear except on Friday when a cloudy sky combined with rain occurred. On this day, the soil and air temperature curves in the upper portion of the figure drop considerably but rise again the next day with clear weather.

Clear days during the summer portion of the 1927 period with the atmosphere calm or with north winds result in increasing the soil temperatures in the surface soil. On the other hand, the opposite effect can be seen on clear days with southerly winds.

As in the previous set of charts, during the winter months the north winds have a cooling effect while the southerly winds have a warming effect on the soil temperatures. Cloudy days in winter have a warming effect on soil temperatures while in summer the opposite is true.

The time of occurrence of the maximum and minimum soil temperatures as compared to the air maximum and minimum varies with the season, character of the sky, etc. As a general rule, the maximum and minimum temperatures at the one-half inch depth occur within one hour after the air maximum or minimum. The lag for the other depths is approximately as follows: 3-inch, 2 hours; 6-inch, 4 hours; 12-inch, 8 hours; 24-inch, 70 hours; and 36-inch, 80 hours.

The distribution of the rainfall during the 1925 and 1927 periods is here shown so that the climatic characteristics of the two periods may be indicated. These data were compiled from the United States Department of Agriculture, Weather Bureau climatological data for those years.

TABLE 1

MONTHLY PRECIPITATION FOR 1925 PERIOD WITH DEPARTURES FROM THE NORMAL,
DAVIS, CALIFORNIA

February		March		April		May		June		July		August		September	
Precipitation	Departure														
4.28	+1.47	3.10	+0.69	2.15	+1.06	1.63	+1.00	0.02	-0.12	0	-0.01	0.03	+0.02	0.10	-0.23

During the 1925 (February to September inclusive) period as shown in table 1 the total precipitation was 11.31 inches which was 3.88 inches above normal. This is likely to create an erroneous application to the soil temperatures unless one notes the character of the rainfall. The first figure for the 1925 season is for the week of February 23 (fig. 1). There was no rainfall from February 23 until March 28, as shown in figure 5. On March 31 (fig. 6) there was

TABLE 2

MONTHLY PRECIPITATION FOR 1927 PERIOD WITH DEPARTURES FROM THE NORMAL,
DAVIS, CALIFORNIA

December		January		February		March		April		May		June	
Precipitation	Departure												
0.58	-2.82	2.18	-1.69	4.66	+1.85	1.07	-1.34	2.48	+1.39	0.31	-0.32	0.47	+0.33

a recorded rainfall of 2.50 inches which brought the rainfall for March to 0.69 above normal. As the period of February 23 to March 28 was without rain, the soil temperature curves have distinct peaks and depressions.

In like manner the precipitation data is shown for the second period starting with December 1926.

During the 1927 period as shown in table 2 there was a total precipitation of 11.75 inches which was 2.60 inches below normal for the months of December-June inclusive. A study of the daily or weekly distribution of the rainfall of the figures for the 1927 period will show that up to the week of April 18 (fig. 49) the rainfall, although below normal except for February, was well distributed—frequent light rains. After April 15 in this period, to May 20 (fig. 53) there was no recorded rainfall. It is clearly evident from the above that one must consider the daily or hourly distribution of the rain instead of the monthly amounts only, in order to note the effect of this climatic element on the soil temperatures.

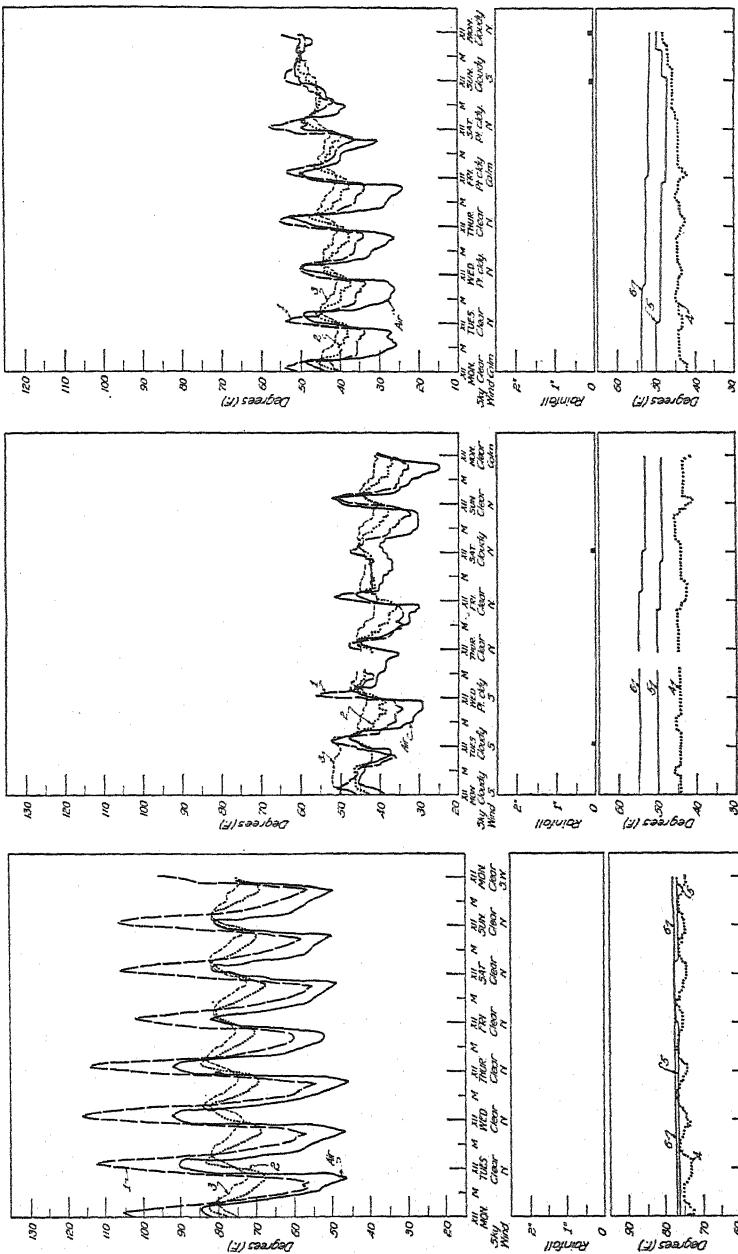


Fig. 31. Week of September 21, 1925
Fig. 32. Week of December 20, 1926
Fig. 33. Week of December 27, 1926
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

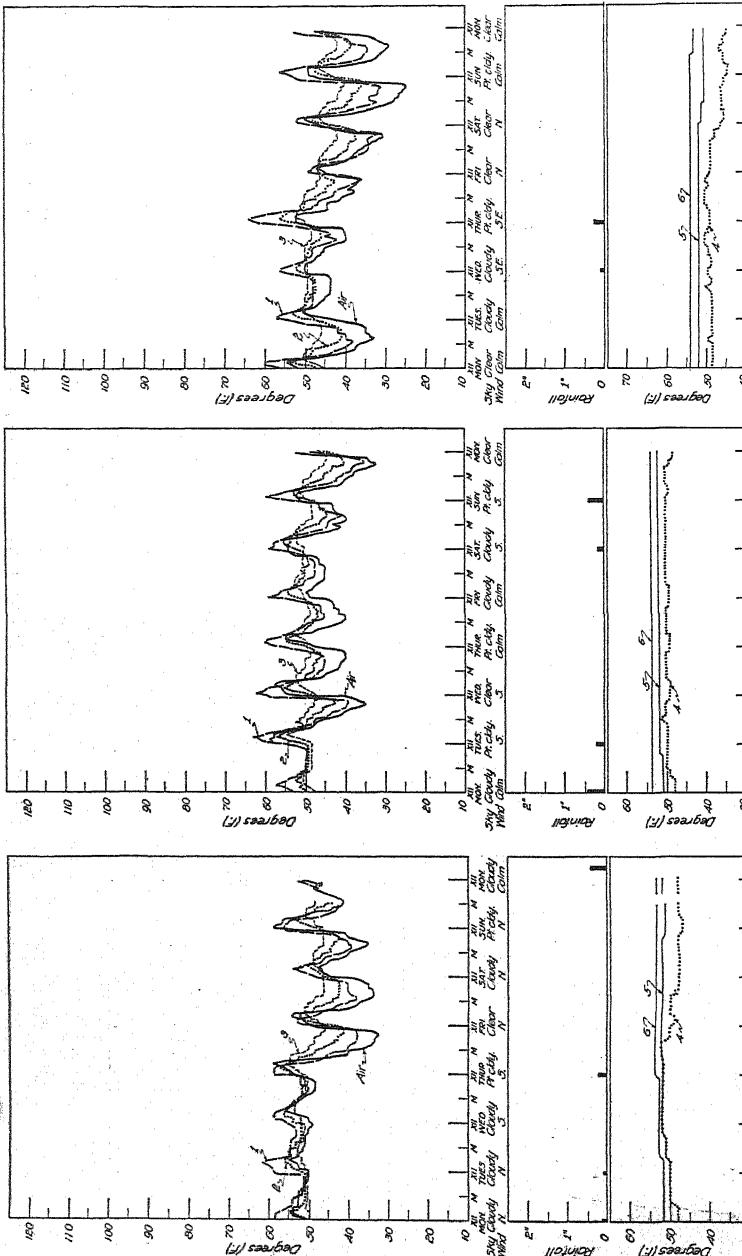


Fig. 34. Week of January 3, 1927

Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 35. Week of January 10, 1927

Fig. 36.—Week of January 17, 1927

Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

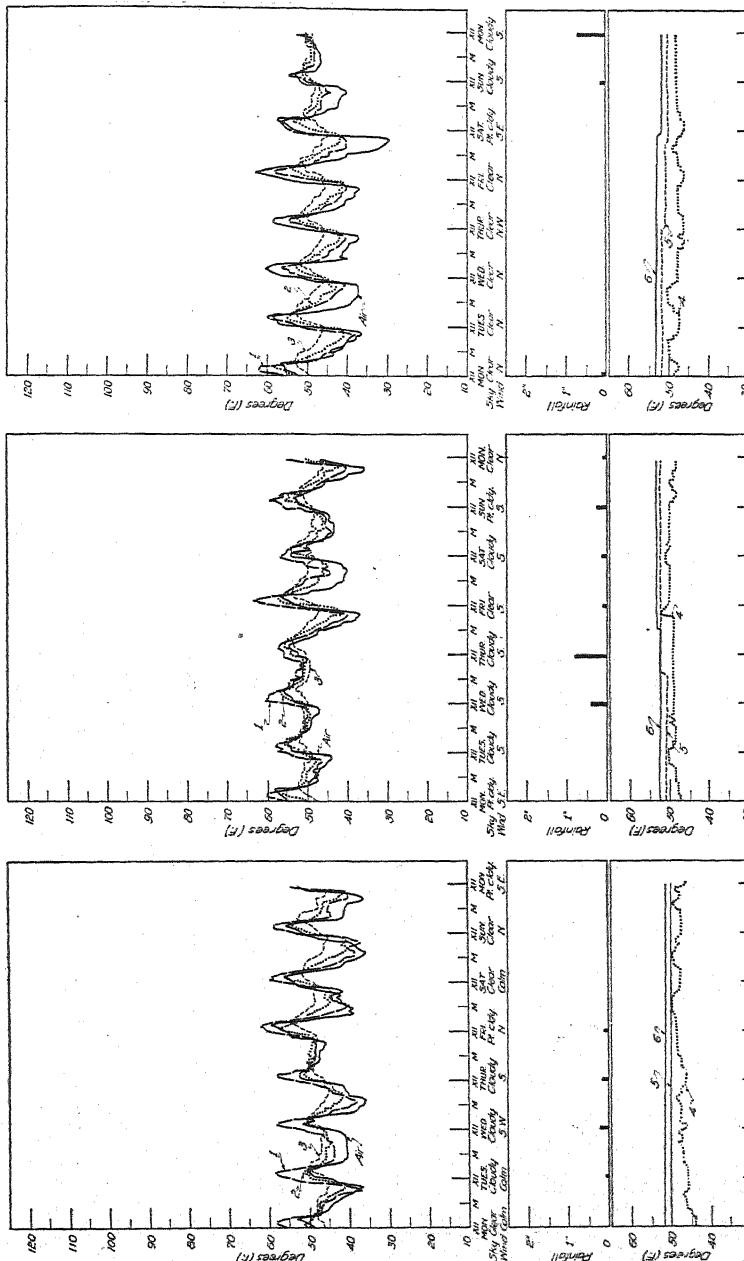


Fig. 37. Week of January 24, 1927

Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 38. Week of January 31, 1927

Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

Fig. 39.—Week of February 7, 1927.

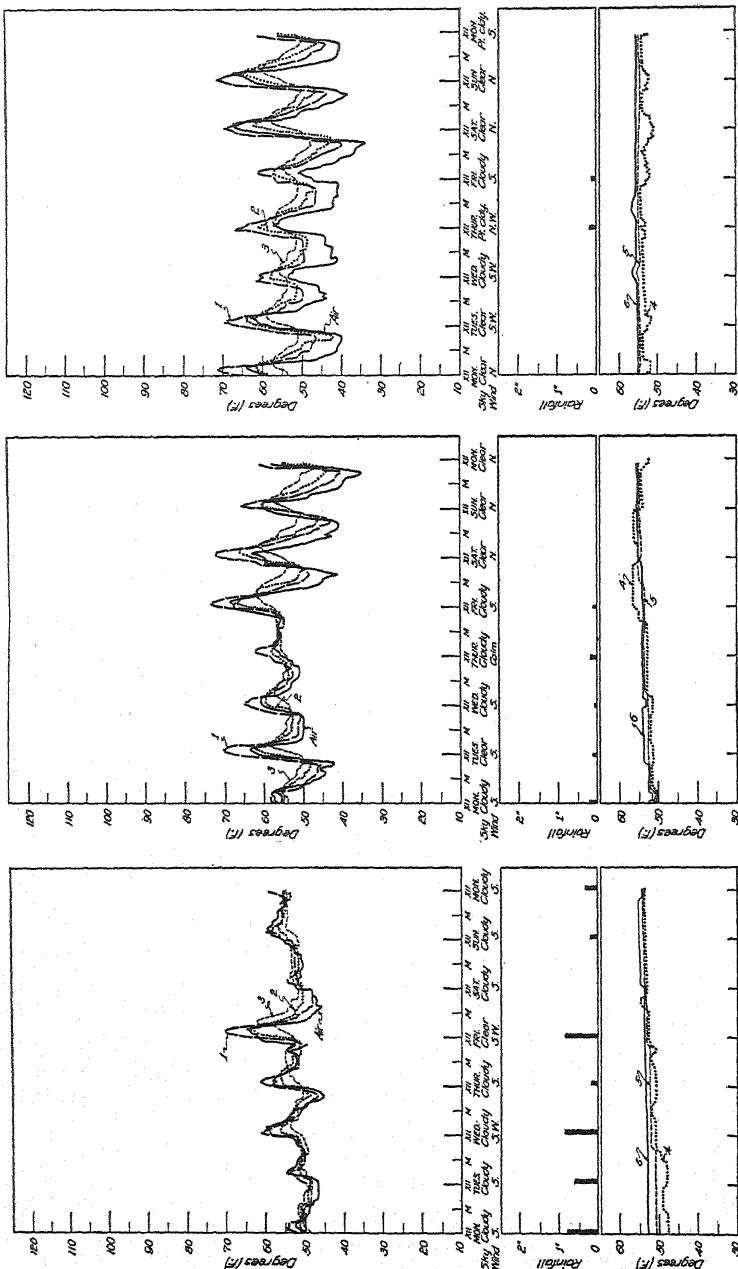


Fig. 40. Week of February 14, 1927
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 41. Week of February 21, 1927

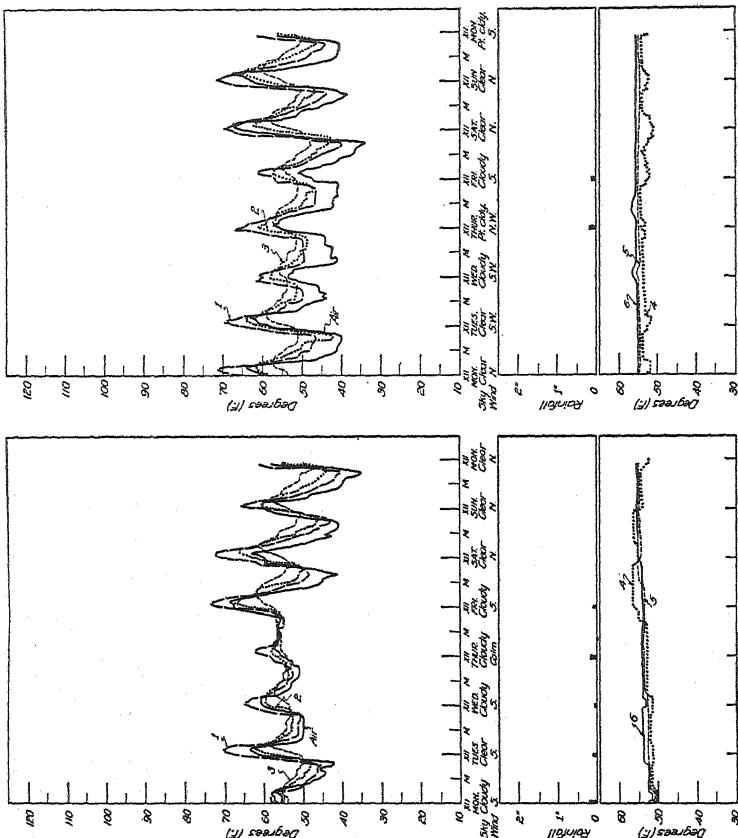


Fig. 42.—Week of February 28, 1927

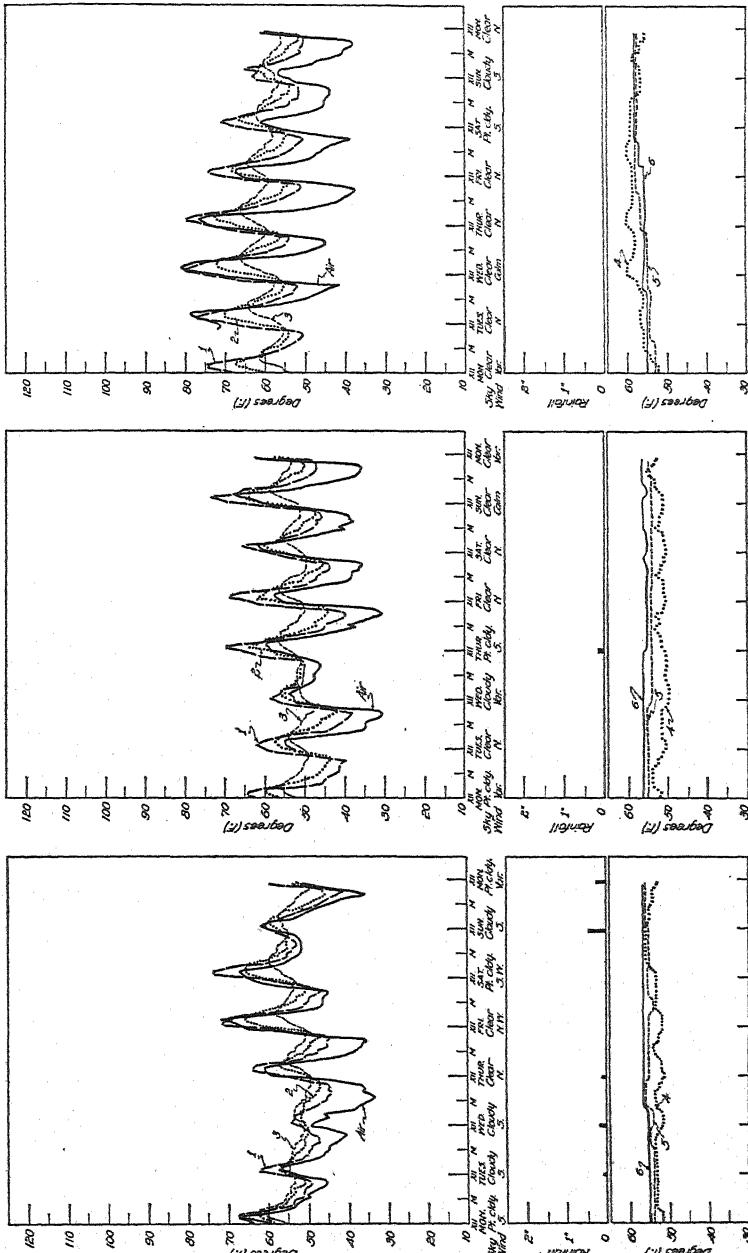


Fig. 43. Week of March 7, 1927
Fig. 44. Week of March 14, 1927
Fig. 45.—Week of March 21, 1927.
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 45.—Week of March 21, 1927.

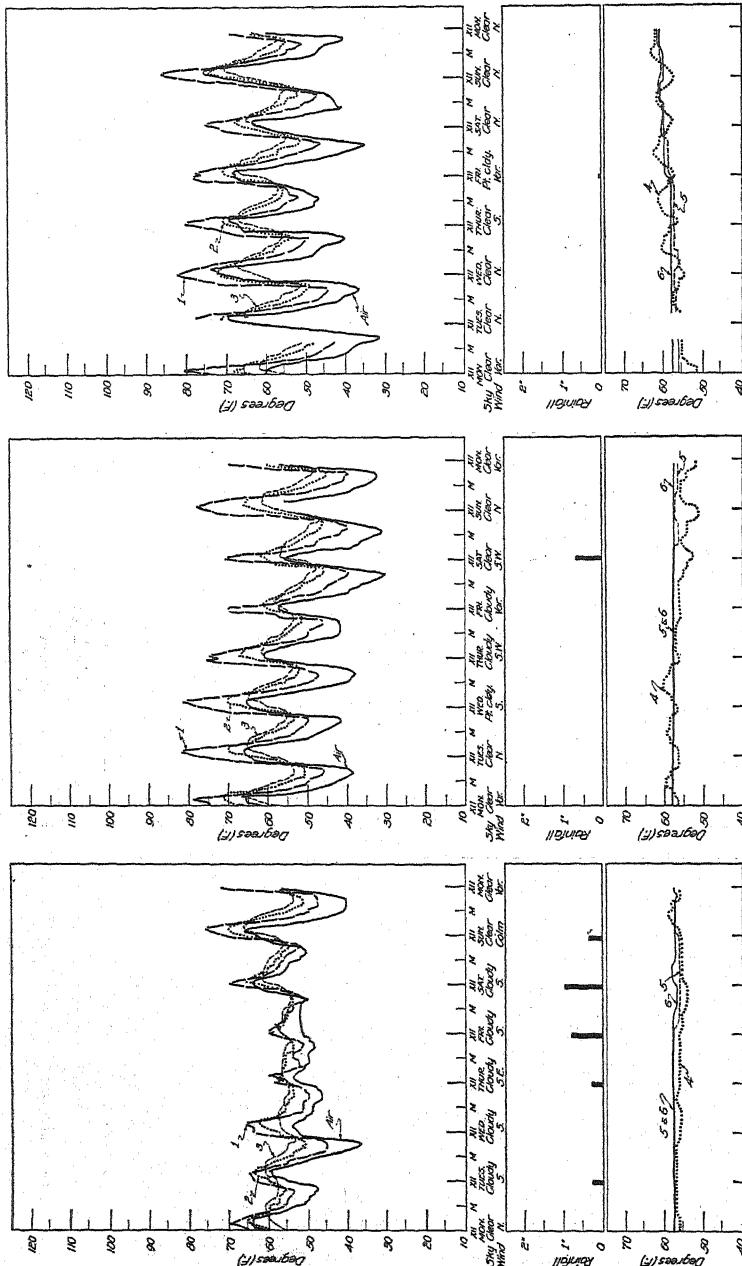


Fig. 46. Week of March 28, 1927
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;
3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 47. Week of April 4, 1927
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3;

Fig. 48.—Week of April 11, 1927.

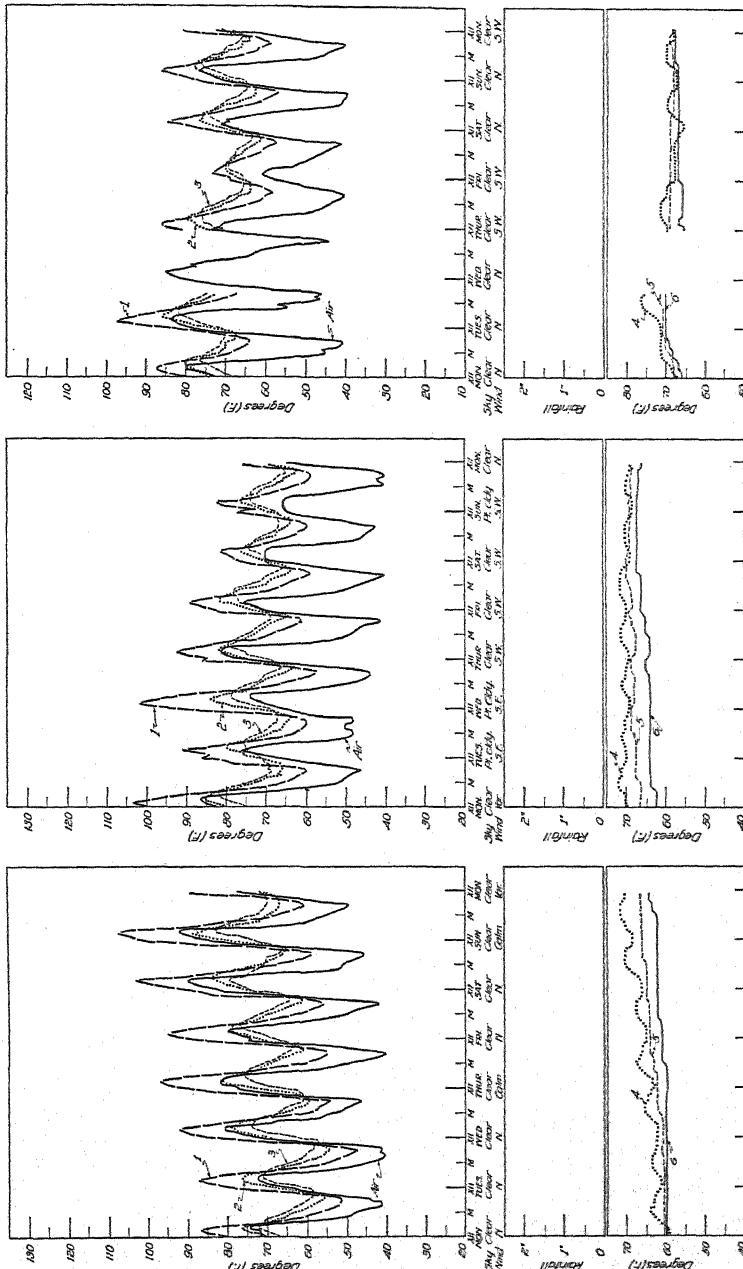


Fig. 49. Week of April 18, 1927
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 50. Week of April 25, 1927

Fig. 51. Week of May 2, 1927

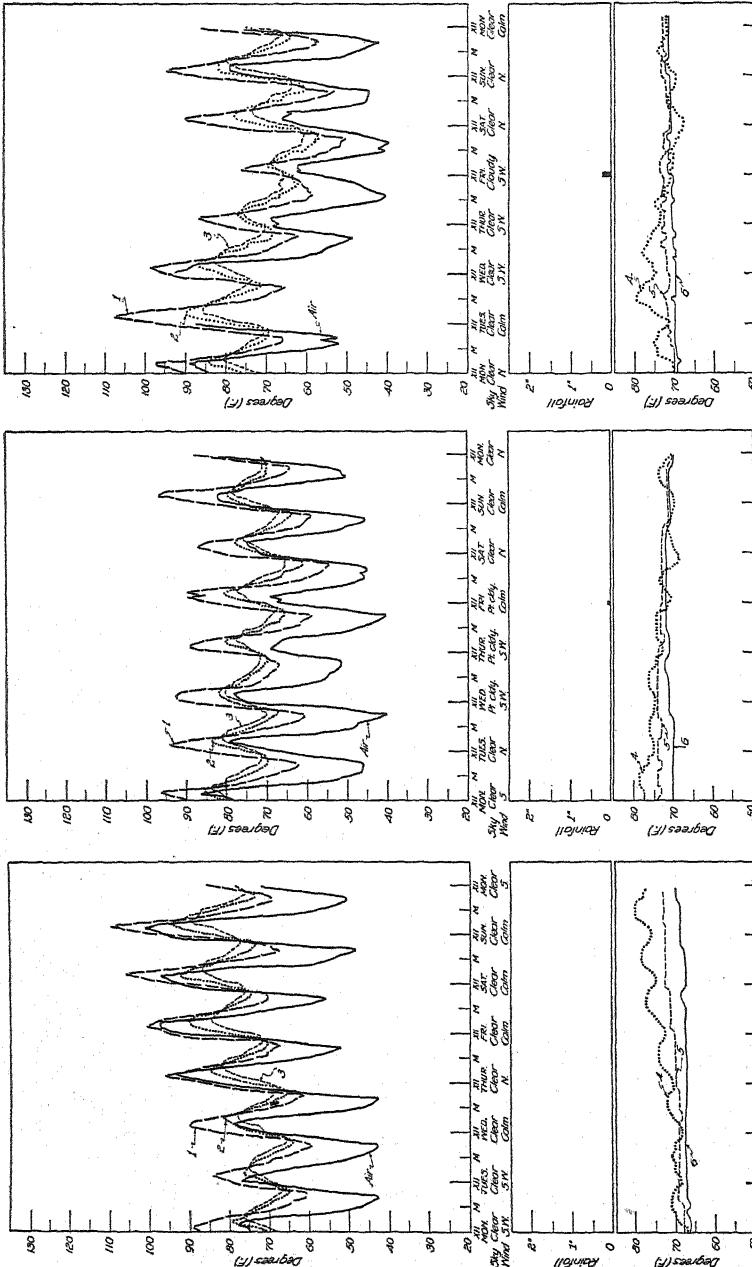


Fig. 52. Week of May 9, 1927
Soil and air temperatures and atmospheric conditions. Curve 1 shows soil temperature at one-half inch depth; 2 at 3; 3 at 6; 4 at 12; 5 at 24; and 6 at 36-inch depths.

Fig. 53. Week of May 16, 1927

Fig. 54.—Week of May 23, 1927.

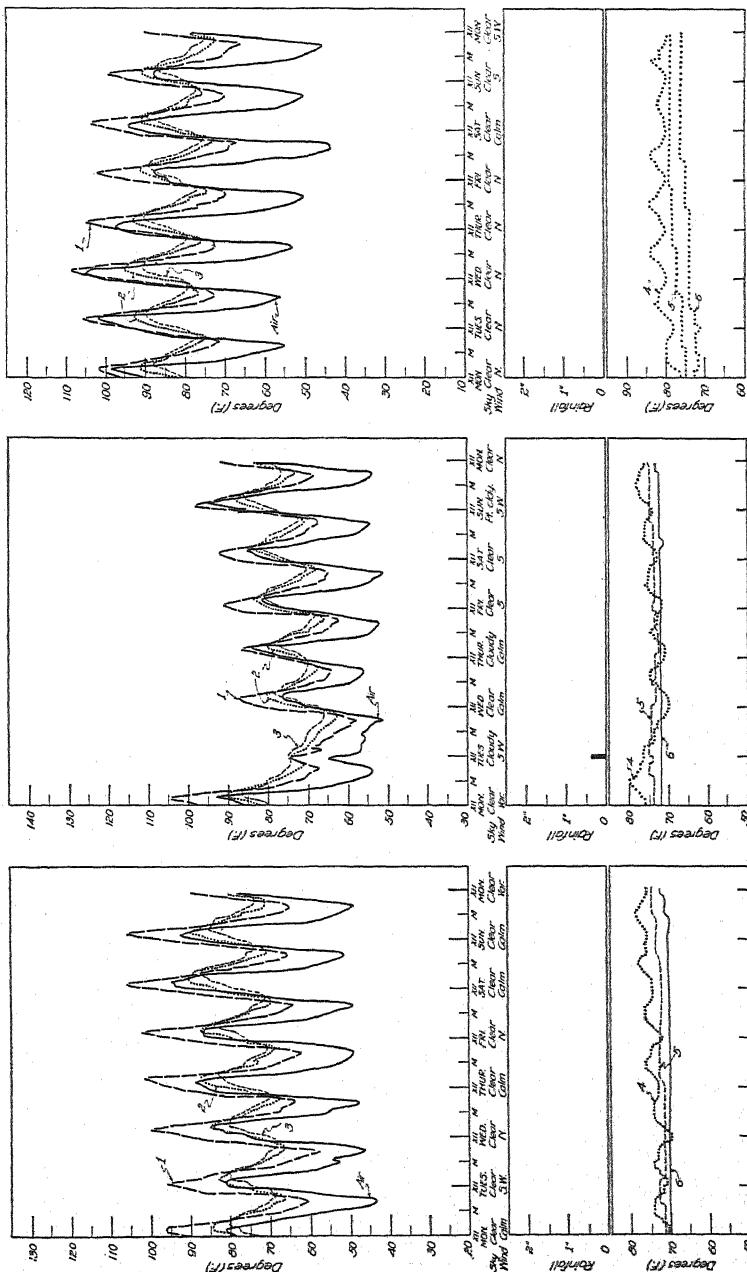


Fig. 55. Week of May 30, 1927

Fig. 56. Week of June 6, 1927

Fig. 57.—Week of June 13, 1927.
No. of one-half inch dents: σ at 2.

SUMMARY

The moisture content of the surface 3 feet of loam was 20-22 per cent, while the subsoil, of fine sandy loam, was 16-18 per cent, after a seasonal rainfall totaling about 3 inches which replenished the moisture lost by evaporation during the preceding season. The moisture equivalent of the loam is around 20, and for the fine sandy loam, it is 16. No crops were grown on this area after 1923 and the weeds were kept down by cultivating once a month. During the dry season, the moisture content of the surface 4 inches was reduced to approximately 5 per cent. The next depth, 4-8 inches, contained about 12 per cent, and the deeper layers of the loam contained less. In the last foot of soil, 66-72 inches, the moisture content was about 15 per cent. The changing moisture content was due almost entirely to vapor movement. Where an unperforated black paper mulch had been placed on the soil surface, the moisture content in the surface 4 inches was about 20 per cent, while in the other depths below the surface 4-inch layer, the moisture content during the dry season was practically the same as in the cultivated area not covered with paper mulch.

The temperature changes occurring at the one-half inch, 3, 6, 12, 24 and 36-inch depths as well as the air temperature are shown for the period of February 23, 1925 to September 28, 1925 by hourly intervals. During these months many California crops are seeded and harvested and would be subjected to somewhat similar conditions of "soil climate."

The range in the air temperatures during the 1925 period was 86° while at a depth of one-half inch the soil temperature range was 114°. The range for the deeper soil areas was progressively smaller so that at the 36-inch depth, it was the least, or 31°.

The effect of the character of the sky, rainfall, and wind during the 1925 period are indicated in certain graphs more clearly than in others. The diurnal range is influenced by these climatic elements and during this period it was less than 2° at the 36-inch depth and up to 66° at the one-half inch depth.

The temperature changes occurring at the one-half inch 3, 6, 12, 24, and 36-inch depths as well as the air temperatures are shown for a second period, December 20, 1926 to June 20, 1927 by hourly intervals. These conditions would be met in general by the winter-growing California crops seeded and harvested during this period.

The range in air temperatures during the 1927 period was 81° while at a depth of one-half inch it was 76° . The lowest air temperature recorded was 24° while the lowest soil temperature at the one-half inch depth was 32° —or 8 degrees higher. The range for the deeper soil layers was progressively smaller so that at the 36-inch depth it was only 24° during this period.

The effect of the character of the sky, rainfall, and wind during the 1927 period are well shown in the graphs. From December 20 to April 18, there was some rainfall each week with one exception. During this moist period the air temperature curve and soil temperature curves for the one-half inch, 3-inch, and 6-inch depths coincide very closely. Cloudiness prevailed during most of this period so that the diurnal temperature range was very small for these depths.

The time of the occurrence of the maximum and minimum soil temperatures as compared to the maximum and minimum air temperatures, or the lag, varies from less than one hour at the one-half inch depth to approximately eighty hours at the 36-inch depth.

The effect of the daily distribution of the rainfall on the soil temperatures is very marked and during years when the monthly rainfall is recorded as being above normal, the soil temperatures during that month may show a very distinct rise and fall on account of the fact that the rainfall may have been confined to a very short period.

The various parts of plants growing in the field, such as the leaves, stems, and roots, are subjected to widely different temperature conditions. On July 17, 1925, the leaves and branches were in air heated to 116° ; the stem (unshaded) just below the ground surface was in soil having a temperature of 143° ; while the roots were in a medium with a temperature of 107° at the 3-inch depth to 84° at the 24-inch depth.

The possible influence of soil temperatures on the activity of bacteria, fungi, available nitrogen, chemical reactions taking place in soils, diffusion of the dissolved material through the roots and plant tissues and the rate of decomposition in soils is indicated by the seasonal temperature changes occurring at various depths in soils.

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ROOT DEVELOPMENT AND SOIL MOISTURE

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INTRODUCTION

The root development of plants and its relation to the amount and availability of soil moisture have been the subject of much study and speculation. Observations in California have yielded results which differ from conclusions drawn from some studies of similar nature elsewhere. Climatic conditions prevalent in California afford excellent opportunity for such study because the effective rainfall occurs almost entirely during the winter months and because soil-moisture conditions during a summer growing season are, in consequence, largely under control.

Where the water table is far from the surface, experiments in California⁽¹⁴⁾ have shown that the capillary movement of moisture is too slow to meet the needs of growing plants. Naturally then, roots must extend into a body of soil to utilize its moisture. Under these conditions, furthermore, direct evaporation causes material loss of moisture from only shallow depths of the soil, and moisture below 8 inches is lost by evaporation at an extremely slow rate, while plant transpiration accounts for the greater part of the water loss below this depth. It was, therefore, suggested⁽¹⁴⁾ that the results of soil-moisture determinations, if made on adequate samples properly timed, would indicate the presence or absence of roots of plants growing on the soil. With soil previously wet, relatively dry soil below the surface layer would indicate the presence of roots. This paper presents some data in support of this suggestion. While it has to do with the development of the roots of grain-sorghum plants in relation to soil moisture, it is thought that the results obtained justify wider application.

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SOIL MOISTURE DETERMINATIONS

Because of variations in soil texture between the different samples, Briggs and McLane⁽⁵⁾ and Alway, McDole, and Trumbull⁽²⁾ have pointed out the need of expressing soil moisture as a ratio. The former investigators used the moisture equivalent, while the latter took the hygroscopic coefficient as the basis of comparison. Puri⁽¹²⁾ has shown, however, that the hygroscopic coefficient cannot be determined satisfactorily, and Linford⁽¹⁰⁾ has further demonstrated that there cannot be equilibrium between relatively dry soil and saturated vapor, as many investigators have assumed. The hygroscopic coefficient, which has been considered an equilibrium point by some workers, is, therefore, of doubtful value for use in evaluating the moisture content of soils. On the other hand, moisture-equivalent determinations, when made in accordance with an exact procedure, have proved⁽¹⁶⁾ to give reproducible results and may be used with confidence. For these reasons, the exclusive use of the moisture equivalent as a measure of the relative moisture retentiveness of soils strongly suggests itself.

Sedimentary soils are generally stratified, with the different layers often varying widely in texture from one another. Sand, gravel, and other variations in soil texture may occur as isolated pockets in any one layer, making the soil still more variable. The surfaces between strata are, furthermore, often irregular. In samples taken under such conditions, the soil from one hole at a given depth may vary in texture from that secured from an adjacent hole. For these reasons, a moisture-equivalent determination has been made on each sample taken in this study. This is a very desirable procedure to follow in all field studies directly bearing on the relation of soil moisture to plant growth, and may be necessary for the proper interpretation of results when relatively few samples are taken.

A typical case is illustrated in the results obtained in an intensive soil-moisture study at Davis, California. The soil of the particular area which was being investigated is Yolo silt loam. An intensive soil survey made according to standard practice had not indicated that this soil was exceptionally non-uniform to a depth of 6 feet. On January 17, 1927, samples by one-foot increments to a depth of 9 feet were taken with a specially designed soil tube⁽¹⁵⁾ from 6 holes, five of which were located within a circle 12 inches in diameter, the sixth being at the center of the circle. The holes were, therefore, only about 4 inches apart, and were as close as possible to each other without interfering. Moisture determinations were made in the usual way, and moisture

equivalents were run on each sample by the procedure described in a citation previously mentioned.⁽¹⁶⁾ The ratio of the moisture content to the moisture equivalent of each sample was calculated and expressed in percentage. For the sake of convenience, this ratio will be referred to as 'relative wetness'; in the same way Alway, McDole, and Trumbull⁽²⁾ have suggested the term 'relative moistness' for the ratio of moisture content to the hygroscopic coefficient. The moisture percentage, moisture equivalent, and relative wetness of the samples are given in table 1.

TABLE 1

MOISTURE CONTENTS,* MOISTURE EQUIVALENTS, AND RELATIVE WETNESS OF
SAMPLES OF SILT LOAM SOIL TAKEN WITHIN A CIRCLE ONE FOOT IN
DIAMETER. DISTANCE BETWEEN HOLES ABOUT 4 INCHES.
JANUARY 17, 1927, DAVIS, CALIFORNIA

Hole	Depth of soil sampled, in feet									
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	Mean
MOISTURE CONTENT										
1.....	25.3	23.6	15.6	11.0	11.0	9.3	11.6	13.7	16.9	15.3
2.....	25.9	23.9	16.8	11.2	9.9	9.4	11.7	14.4	16.4	15.9
3.....	24.6	23.2	17.2	12.0	10.0	9.0	11.2	15.2	15.3	15.3
4.....	25.8	23.8	17.4	11.3	9.8	9.1	12.2	15.1	14.7	15.6
5.....	25.4	23.3	13.5	10.9	9.4	9.0	13.8	13.1	14.1	14.4
6.....	25.6	23.1	19.5	11.8	10.4	9.2	11.8	13.9	15.9	15.7
Mean.....	25.4	23.5	16.7	11.4	10.1	9.2	12.0	14.2	15.6	15.3
Standard deviation.....	0.47	0.33	2.00	0.44	0.55	0.02	0.29	0.83	1.05
Coefficient of variability.....	1.9	1.4	12.0	3.9	5.4	0.2	2.4	5.8	6.9
MOISTURE EQUIVALENT										
1.....	24.0	22.6	19.7	18.5	18.4	14.0	16.7	21.0	24.3	19.9
2.....	24.5	21.4	18.6	17.8	15.4	13.0	15.8	20.3	22.4	18.8
3.....	23.6	21.8	18.2	18.6	14.7	12.1	17.2	22.2	20.2	18.7
4.....	24.1	23.0	18.1	16.9	13.7	11.6	15.6	22.6	20.2	18.4
5.....	23.6	22.6	14.9	16.3	12.5	11.7	17.8	22.3	18.7	17.8
6.....	24.1	21.0	21.0	17.9	16.2	13.0	16.5	19.8	20.6	18.9
Mean.....	24.0	22.1	18.4	17.7	15.1	12.6	16.6	21.4	21.1	18.8
Standard deviation.....	0.34	0.79	2.06	0.91	2.04	0.93	0.83	1.15	1.98
Coefficient of variability.....	1.4	3.6	11.2	5.1	12.7	7.4	5.0	5.4	9.4
RELATIVE WETNESS										
1.....	106	104	79	60	60	66	70	65	70	76
2.....	106	112	90	63	64	72	74	71	73	81
3.....	104	106	95	64	68	74	65	68	76	80
4.....	107	104	96	67	71	78	78	67	73	82
5.....	108	103	91	67	75	77	77	59	75	81
6.....	106	110	93	66	64	71	71	70	77	81
Mean.....	106	106	91	64	67	73	72	66	74	80
Standard deviation.....	1.3	3.6	5.8	2.8	5.4	4.4	4.9	4.3	2.5
Coefficient of variability.....	1.3	3.4	9.4	4.4	8.1	6.0	6.8	6.4	3.4

* Moisture contents and moisture equivalents are percentages on a dry-weight basis. Relative wetness is the ratio of moisture content to moisture equivalent expressed as percentage.

The variations in moisture equivalents are especially important, for they reflect the variations in soil texture at the same depth. These samples were only a few inches apart. In fact, they were as close as it would be convenient to take them to the depth indicated. With a greater distance between holes, a greater difference in soil texture might be expected. While the moisture content of the soil in the depths 0 to 1, 1 to 2, and 5 to 6 feet in the 6 holes did not vary any more than might be expected, the variations in the other depths are surprisingly great when the closeness of sampling is considered. It has been shown elsewhere⁽¹⁴⁾⁽¹⁸⁾ that the moisture equivalent of the soils of the Yolo series at Davis is a fair measure of the maximum field capacity of these soils. Although the difficulties in determining the moisture contents and moisture equivalents make it seem probable that such work may sometimes involve a percentage of error of as much as 10, in the ratios of moisture content to moisture equivalent the soil within the 1-foot circle tested was obviously wet to not quite 3 feet in depth. The soil was sampled the day after heavy rains had ceased. The figure 79 for the relative wetness in the third foot of hole 1 (table 1) would indicate that the rain had not penetrated so deeply there as in the rest of the area.

A procedure sometimes used in soil-moisture field studies bearing on plant growth involves the determination of moisture equivalents on but a very small proportion of the samples taken. From experience gained in this and other studies, the writers feel that in soil that is not unusually uniform, such a procedure may lead to erroneous conclusions unless the differences due to variation in texture can be minimized by taking a considerable number of samples for each condition investigated.

SOIL-MOISTURE CONDITIONS AFTER MATURITY OF GRAIN SORGHUM

The methods of sampling and expressing the results as outlined above were used in a field study of the relation of soil moisture to root development. Several strips of White Yolo, a grain sorghum, were planted during the first week in May, 1923, across a field which had been clean-cultivated the previous season, leaving strips of unplanted land. Because of the absence of competition on one side, the outside row next to the unplanted, clean-cultivated land grew much better than the inside rows. Soil samples were taken with the soil tube previously mentioned, on September 18 and 19, 1923, in one-foot

increments to a total depth of 6 feet. Thirteen holes were made, each approximately one foot apart, beginning at the second row of sorghum from the edge of the unplanted land, and continuing across the first row and 8 feet into the unplanted land on a line at right angles to the row. Moisture determinations were made on these samples in the usual way, and moisture equivalents were run in duplicate on each of the oven-dried samples. The mean of the two moisture-equivalent determinations was used to calculate the relative wetness of each sample.

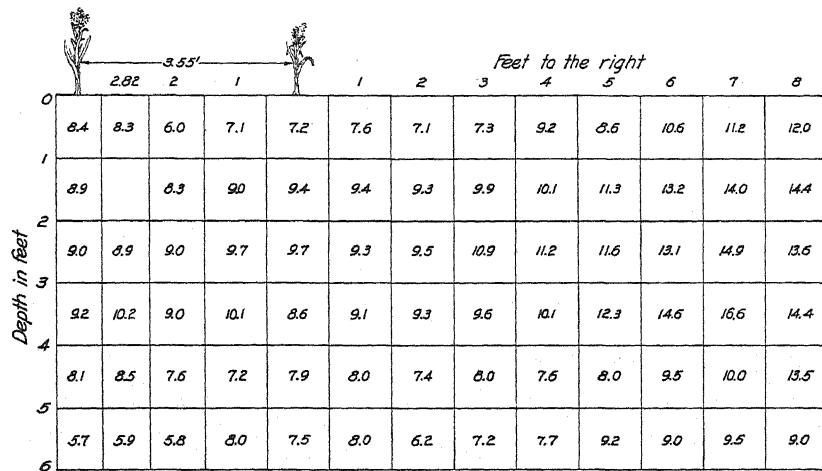


Fig. 1. Moisture contents of soil after sorghum plants have matured. The soil to the right of plant rows was kept free of all vegetation.

Figure 1 shows the percentages of moisture found. These, and all other moisture data presented in this paper, are calculated on the basis of weight of oven-dried soil. The moisture percentages, alone, convey no accurate idea as to which portions of the soil mass were wet and which were dry. With their respective moisture equivalents, however, these figures gain interest. There is close agreement between the moisture content corresponding to the moisture equivalent and the maximum field capacity of the Yolo soils at Davis; and indeed the work of Alway and McDole,⁽¹⁾ and Burr and Russell⁽²⁾ indicates that this agreement is also fairly close in other localities. The moisture equivalents of the samples obtained in this test may therefore be taken to represent the amount of moisture in the soil after the last rain in the spring and after downward movement had practically ceased.

Figure 2 gives the relative wetness of the respective samples of figure 1, which may be taken to represent the percentage of the initial moisture remaining after the sorghum plants had matured. The plants at this time were not permanently wilted. It is interesting to note that 54 per cent represents the theoretical wilting coefficient as suggested by Briggs and Shantz⁽⁶⁾ when the moisture equivalent is used as the indirect measure of the wilting coefficient. Although it has been found⁽¹⁹⁾ recently that a common factor to calculate the

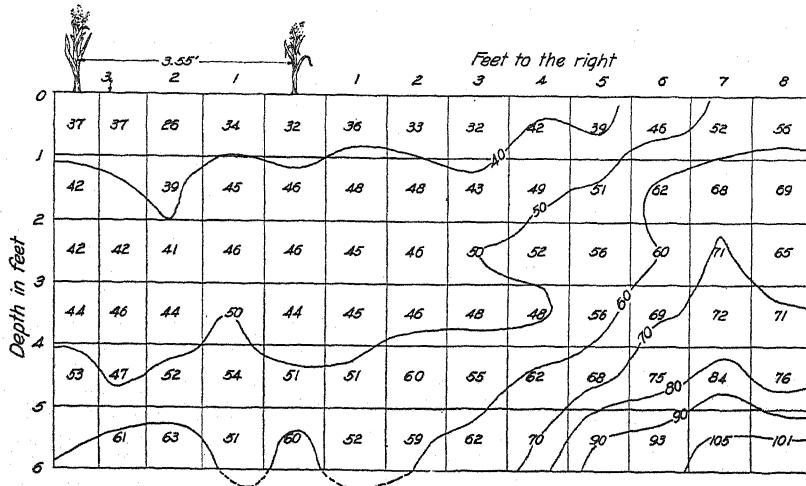


Fig. 2. Moisture conditions of soil after sorghum plants have matured. The irregular curved lines join points of equal relative wetness ($\text{moisture content} \div \text{moisture equivalent} \times 100$).

amount of residual moisture in the soil at the beginning of permanent wilting cannot be used for all soils, the soils of the Yolo series at Davis were found to agree fairly closely with the accepted Briggs and Shantz ratio. While the different Davis soils tested did not agree exactly with each other, the ratio of the residual moisture at permanent wilting to the moisture equivalent averaged about 50 per cent.

The irregular curved lines across the chart in figure 2 were drawn just as contour lines are made on topographical maps, by connecting points of equal relative wetness, starting with the 40 per cent and continuing with 10 per cent intervals, as indicated in the figure. These lines have not been smoothed out, but show all of the errors inherent in the methods and assumptions used. As has been stated before, a percentage error of 10 in the relative wetness may sometimes occur. If these curves were smoothed out into an ideal case, they might be

represented by a series of concentric arcs extending out from the last row into the unplanted area. The center of these arcs would be the crown of the plant. Of course, this ideal situation is modified by the drying out of the surface soil by direct evaporation, which tends to flatten the curves as they approach the surface. The curves of 60, 70, 80, 90, and 100 per cent undoubtedly would have swung under the crown of the plant if samples had been taken to a sufficient depth.

SOIL-MOISTURE CONDITIONS AND ROOT DISTRIBUTION

A second test, similar to that described above, was made at Davis on a piece of unirrigated land which had been cleanly cultivated during 1924. The soil was heavier in texture than that first used and is classed as Yolo clay loam. During the winter of 1924 and 1925, the land was plowed, and in the spring it was worked into a seed bed by shallow surface cultivations. On April 24, 1925, a single row 100 feet long of Double Dwarf milo, a grain sorghum, was seeded on a plot of ground that was kept free from weeds. Throughout the growing season of 1925, the adjacent land also was kept free from weed growth by cultivation and hoeing. The row of sorghum plants under observation paralleled on one side a single row or another variety, which was 29 feet distant. On the other side there was a pasture 36 feet away.

During the second week of October, 1925, a trench was dug to a depth of about 10 feet across the row of sorghum plants at right angles, and was extended 8 feet on one side of the row and 15 feet on the other side. A system of coordinates was marked off on the face of the trench, and samples were taken at the places indicated in table 2. The samples were obtained by driving a steel tube 1-13/16 inches in diameter, into the face of the trench. The soil sampling started on October 20, 1925, a few days after the trench was dug. The soil in the face of this trench had, therefore, dried out to some extent before the samples could be obtained. In order to avoid errors that might have resulted from this surface drying, the first 4 inches of soil from each hole was discarded, and the soil from the next 4 or 5 inches in each hole was taken for the samples for moisture determinations.

These samples were placed in weighing cans and moisture determinations made in the usual manner. After the soil had been oven-dried and weighed a 30-gram sample for a moisture-equivalent determination was taken from each can. All roots were removed from the moisture-equivalent sample by sieving and returned to the original

TABLE 2
MOISTURE CONTENTS, MOISTURE EQUIVALENTS, AND AMOUNT OF ROOTS FROM SAMPLES TAKEN FROM A TRENCH UNDER AND ACROSS
A SINGLE ROW OF SORGHUM PLANTS

Depth of soil sampled	Moisture equivalent	Center			1 foot to right			3 feet to right			5 feet to right		
		Dry soil in sample	Milligrams of roots	Milligrams of roots in 100 grams soil	Moisture	Moisture equivalent	Relative wetness	Dry soil in sample	Milligrams of roots	Milligrams of roots in 100 grams soil	Moisture	Moisture equivalent	Relative wetness
0.5	11.3	27.5	41	210.70	4.5	2.14	10.8	27.8	39	193.80	-0.50*
1.5	12.8	26.0	49	160.00	6.0	3.75	12.9	26.2	49	199.55	4.7	2.36	0.81
2.5	12.2	25.1	48	184.10	5.7	3.10	13.5	25.7	51	184.15	1.5	0.31	6.36
3.5	12.3	26.4	47	180.95	9.5	5.25	15.1	29.7	51	173.20	11.0	0	2.54
4.5	14.3	28.2	50	206.20	5.3	2.67	14.0	29.0	48	212.80	5.4	2.4	1.14
5.5	11.8	24.8	48	218.60	1.4	0.64	12.2	26.4	46	210.20	2.4	0.82	0.50
6.5	14.2	25.6	56	231.25	3.8	1.64	15.0	26.6	59	244.70	2.0	0.72
7.5	16.5	27.3	60	269.75	3.9	1.45	17.0	27.3	62	259.15	1.8	0.72
2 feet to right												
0.5	11.8	27.2	43	233.60	1.1	0.47	11.8	26.6	44	223.60	-0.50
1.5	13.1	27.0	49	196.60	2.1	1.07	14.2	27.0	52	169.15	3.5	2.07	3.47
2.5	13.7	26.4	52	198.85	4.8	2.41	13.4	25.6	53	169.85	5.9	3.47	2.72
3.5	14.8	26.9	53	176.90	3.6	2.03	16.6	32.8	51	184.10	5.0	0	2.72
4.5	13.3	27.1	49	181.30	4.0	2.20	13.6	25.4	54	209.75
5.5	13.7	26.3	52	221.65	2.2	0.99	14.9	26.6	56	226.10	7.0	3.09
6.5	14.4	25.5	56	249.40	-0.50	14.6	26.3	55	255.90	1.8	0.72
4 feet to right												
0.5	11.5	26.0	44	188.65	-0.50	15.0	27.0	56	229.55	0.6	0.26	0.32
1.5	15.6	26.2	60	212.25	3.2	1.50	18.3	27.0	68	186.80	0.6	0.32	2.83
2.5	14.1	25.4	56	166.40	3.9	2.35	14.8	24.2	61	173.30	4.9	2.11	2.22
3.5	15.5	27.6	56	197.55	3.7	1.87	17.9	26.9	67	184.90	4.1	2.15	2.15
4.5	14.8	25.2	59	200.50	3.4	1.70	19.4	27.7	70	186.10	4.0	2.15	2.24
5.5	16.9	28.2	60	224.45	12.8	5.67	17.8	27.8	64	201.00	4.5	2.24	1.93
6.5	13.6	25.1	54	217.25	2.4	1.10	13.9	26.0	54	217.45	4.2	0.72	1.93

* -0.50 is used to indicate that the sample contained less than 0.50 milligrams of roots to 100 grams of soil.

TABLE 2 (continued)

TABLE 2 (concluded)

samples. Then roots which were visible to the unaided eye were removed as completely as possible from all of the samples. The larger roots were picked out with forceps, and the soil was washed through sieves of decreasing sizes, the roots which remained on the sieves being removed with a small brush, and adhering soil particles being separated. As all of the work of removal of the roots was done by one operator, the same care was given to each sample.

The results of the samplings are given in table 2 and in figure 3, which also shows the lines of equal relative wetness, as well as those indicating the distribution of the roots. The lines of relative wetness and the lines connecting points having the same amount of roots to 100 grams of soil were drawn in the same manner as the relative wetness lines in figure 2.

TABLE 3
RAINFALL, IN INCHES, AT DAVIS, CALIFORNIA

Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1922.....	2.29	5.85	1.47	0.40	0.40	0	0	0	0	1.56	3.29	7.37	22.63
1923.....	2.62	0.70	0	2.22	0.10	0	0	0	0.35	0.40	0.53	0.88	7.80
1924.....	2.46	2.76	1.18	0.38	0.05	0	0	0	0	2.05	1.42	3.55	13.85
1925.....	1.05	4.28	3.10	2.15	1.63	0.02	0	0.03	0.10	0.10	1.71	1.29	15.46

The rainfall at Davis during 1922, 1923, 1924, and 1925, is given in table 3. From September 1, 1923, to September 1, 1924, 8.99 inches of rain fell, while the rainfall from September 1, 1924, to April 24, 1925 (the date the sorghum was planted) was 17.60 inches. There was only 1.67 inches of rain during the time the plants were growing. As the plot of ground used in this test was kept clean of all vegetation during 1924, a large portion of this rainfall was probably retained in the soil. The soil was thus doubtless wet to a depth of at least 7 feet at the time the row of sorghum was seeded. Although no record is available of the moisture condition of the soil at this time, experience with the soils at this station shows that in years of normal rainfall the soil is wet to a depth of at least 7 feet at the end of the rainy season; it is therefore safe to assume that below the surface mulch, the relative wetness of the soil to a depth of 7 feet was about 100. The results given in table 2 and figure 3 may be taken to indicate the percentage of the amount of the initial water (present in the soil when the plants were seeded) which remained after they had matured. The moisture equivalents given in table 2 indicate that the soil in the plot used in this test was much more uniform in texture than that in the area first tested.

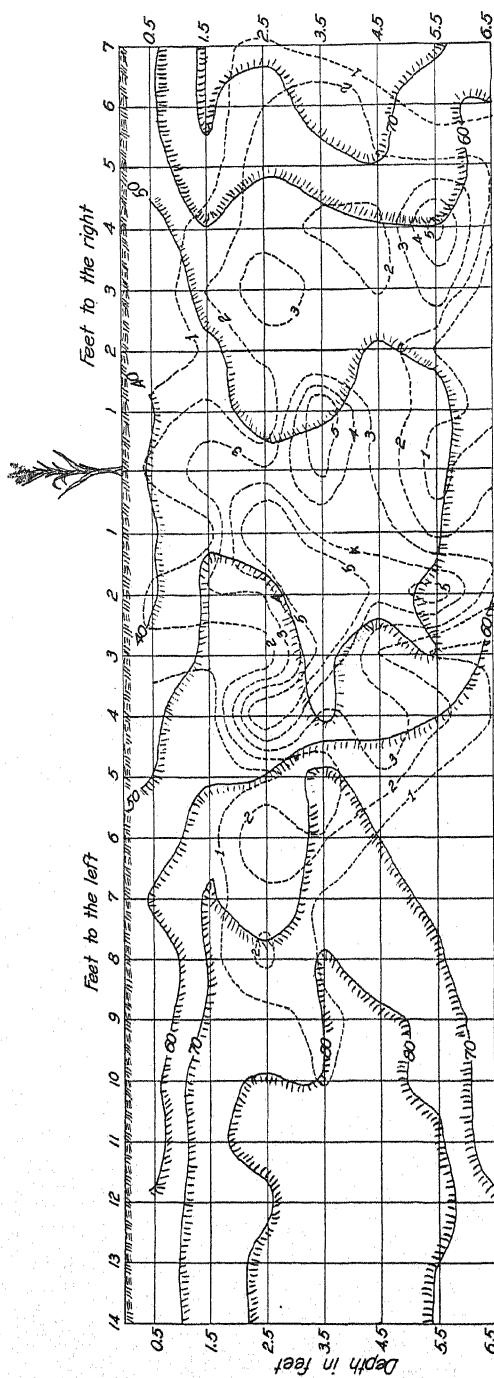


Fig. 3. Soil moisture conditions and distribution of roots after sorghum plants have matured. The solid irregular curved lines join points of equal relative wetness ($\text{moisture content} \div \text{moisture equivalent} \times 100$). The dotted curved lines join points having the same weights of roots; the figures 1, 2, 3, 4, and 5 are actual weights of roots in milligrams to 100 grams of soil.

Some of the samples contained such a small amount of roots that no attempt was made to segregate them, and the amount of roots to 100 grams of soil was assumed to be less than 0.50 milligrams. Figure 3 shows that the soil to a depth of 6 inches was relatively dry even at considerable distance from the row of sorghum plants. This drying out was probably due to evaporation of moisture directly from the surface of the soil. Therefore, in working out the correlation between the percentages for relative wetness and milligrams of roots to 100 grams of soil, the samples from the 0.5 foot depth of soil were not used; and those from which the roots were not removed, because of the small amount contained in them, were listed as having less than 0.50 milligrams to 100 grams of soil. With these assumptions, the correlation between the figures for relative wetness and weight of roots to unit weight of soil was found to be $r = -0.582 \pm 0.040$. This coefficient of correlation, with its relatively small probable error, may be considered to be decidedly significant.

Figure 3 illustrates further the relation of distribution of roots to the relative wetness of the soil. It will be seen that a large majority of the roots are enclosed within the line representing the location of relative wetness of 60 per cent. The line which joins points having one milligram of roots to 100 grams of soil is, in fact, the only one which has its greater portion without the line of 60 per cent relative wetness. As has been mentioned before, tests on these Yolo soils at Davis show that the residual moisture at permanent wilting is about 50 per cent of the moisture equivalent. The bulk of the roots is therefore in soil which has been reduced to about the wilting coefficient.

It must be remembered that only weights of roots were obtained in this experiment. Of course, root hairs, a portion of the roots constituting a part of the actual absorbing surface, could not be seen or measured by the methods employed. It is assumed, however, that there is close relation between weight and absorbing surface.

DISCUSSION

Previous investigations⁽¹⁴⁾⁽¹⁸⁾ at this station have shown that the loss of moisture by direct evaporation from the surface of the soil is practically confined to shallow depths of soil, and that losses from deeper layers (aside from percolation when excessive amounts of water are applied) are due to transpiration from plants growing on the soil. There is, furthermore, abundant evidence⁽¹¹⁾⁽¹⁴⁾⁽¹⁸⁾⁽²²⁾ that capillary movement of moisture from moist soil to drier soil, especially

when the soil is not in contact with a free water surface, is too limited in extent and in rate to be effective for use by the roots of plants; and it appears very probable that capillarity cannot be counted on to move moisture appreciable distances from moist soil into soil that has been dried by root extraction.

It is, therefore, evident that an additional moisture supply can be obtained only by the elongation of the absorbing portion of the roots into new moist soil, unless the moisture be returned to the dry soil by rain or irrigation. The shape of the relative wetness lines in figures 2 and 3 indicates that this elongation process took place under the sorghum plants studied. Apparently, the moisture was removed in successive zones. The drying out of the soil below the surface layer which was affected by direct evaporation could be accounted for only by the presence of roots; and, indeed, the data presented in this paper clearly indicate this explanation to be correct. The negative correlation between the relative wetness of the soil and the density of roots shows that the soil was dried because of the presence of roots. It is apparent, then, that the results of soil sampling, made in the manner described in this paper, may be used to indicate the distribution of roots of plants.

Moisture studies⁽¹⁹⁾ made in small containers show that plants will permanently wilt as soon as the moisture reaches a certain percentage, which is roughly 50 per cent of the moisture equivalent in soils at Davis. If a portion of the root system is partly in dry soil[†] and partly in wet soil, the needs of the plants might be adequately met by absorption from the moist soil. A situation of this kind is illustrated in figure 3. Here part of the root system is in dry soil and part in moist soil. In October the transpiration requirements of the plants were being adequately met by these conditions, although the greater amount of the root system was in dry soil. At this station it has been observed sometimes that plants were not showing evidence of lack of water when the upper few feet of soil (which presumably contained the greater portion of the roots) was dry, if the lower layers of soil (in which the ends of the roots were located) were moist. There is no reason to assume that because the greater amount of roots or of absorbing surface may be in the top layers of soil, absorption by the

[†] A remarkable constancy of the residual moisture content for a given soil when permanent wilting is attained has been observed.⁽¹⁹⁾ There appears to be a rather narrow range of soil-moisture percentage, below which plants cannot obtain moisture rapidly enough to permit them to function properly, but above which transpiration needs of the plant seem to be satisfied adequately. In this discussion, soil containing a percentage of moisture below this range is called dry soil, and soil with a moisture content above this range is called moist or wet soil.

smaller amount of roots in the deeper moist layers cannot be sufficient, especially under low evaporation conditions, to satisfy the needs of plants when the top layers become dry.

The question arises as to whether roots may elongate into dry soil when they are supplied with moisture from roots of the same plant in moist soil. Shantz,⁽¹³⁾ says of the trees of the African grasslands: "These drought-resistant plants also have the ability to push their roots into a dry soil, and in this way be prepared rapidly to absorb soil moisture when it comes . . . but ordinary field crops have no such ability." In this connection may be mentioned Livingston's⁽⁹⁾ studies at Tucson, Arizona, with desert plants, which showed that the roots of seedlings elongated directly downward so rapidly as to make it appear possible for them to reach a permanent and adequate water supply before the soil, wet thoroughly by the frequent showers of the rainy season, can produce injury through conditions of drought. The deeper soil layers of the typical locality studied by Livingston were found to contain at the end of the dry season (and, it was assumed, at all times) a water content adequate to the needs of those desert plants which are active throughout the months of drought. On the other hand, there is no evidence in Livingston's report to show that the roots of these plants had the ability to traverse dry soils, a condition which he doubtless would have observed.

With agricultural plants, there seems to be no experimental evidence that roots will be forced through dry soils. While Auchter's⁽⁸⁾ evidence concerning the cross transfer of water in the plant was not conclusive, it did suggest that water may move through or around the plant without much difficulty. According to Auchter's statement his evidence strongly suggests that the foods manufactured on one side of a plant are used and stored mainly in that side, or are translocated to the roots beneath, and that mineral nutrients absorbed by the roots on one side of a plant are translocated to and used by the trunk, limbs, and leaves directly above them. The ready cross transfer of water from roots in moist soil to roots in dry would serve to keep the latter turgid, but unless elaborated foods and mineral nutrients were also carried to them or derived from a previously stored supply, elongation would soon cease.

Taking as a basis the assumption that elongation of the roots of agricultural plants at least will not take place in dry soil, the writers give the following hypothetical case, which they believe would be about as extreme as would normally be found under actual farming conditions. When a plant has exhausted the available moisture from

most of the soil permeated by roots, if frequent light irrigations or rains keep the upper layers of soil moist, leaving the layers below dry, elongation, if it occurs at all, will take place in the moistened top soil. This should result in an increase in the weight of roots in the upper soil over that in the lower. But it does not necessarily follow that if the whole soil mass is wet again, and the top layers dry out by root absorption, the absorptive area in the lower levels will be insufficient to support the needs of the plants.

Some studies bearing on this question have been made by Beckett and Huberty,⁽⁴⁾ under conditions probably not so extreme as that postulated above, but nevertheless with marked variations in irrigation practice and consequently of soil-moisture conditions. Consequently the following description of the experiments taken from their report is given in some detail. At Davis certain plots of alfalfa of about 0.7 of an acre in area, which were planted in 1921, were irrigated for a period of five years, 1921 to 1925, with a total seasonal depth of 30 inches on each plot, but with the number of irrigations varying from 2 to 12. The distribution of frequencies of application and the depths were as follows: twelve 2½-inch; eight 3¾-inch; six 5-inch; four 7½-inch; three 10-inch; and two 15-inch. At Delhi, plots of alfalfa of 1.07 acres were planted in 1921, and all received identical irrigations during that year. During 1922, 1923, 1924, and 1925, each plot received varying amounts of water and different frequencies of applications as follows: six 8-inch irrigations from 1922 to 1925; six 6-inch irrigations, 1922 to 1925; three 4-inch irrigations, 1922 to 1924, and six 6-inch irrigations during 1925; twelve 3-inch irrigations, 1922 to 1924, and four 6-inch irrigations during 1925.

The soil at Davis on which the plots were located is a Yolo fine sandy loam with an average maximum field capacity of from 20 to 22 per cent. The depth to the ground water level was greater than 14 feet throughout the five years of the experiment. The plots at Delhi were on Oakley fine sand which has a maximum field capacity of about 6 per cent at the surface, and 10 per cent and 12 per cent at the lower depths. A hardpan layer practically impervious to water, 10 to 12 inches in thickness, underlies the area at depths of 6 to 9 feet. The water table under the Delhi plots was always more than 25 feet from the surface during the period of observation.

Beckett and Huberty separated the roots from the soil in 6-inch layers, weighed them, and recorded their distribution to a depth of 6 feet. They concluded that "when the winter rainfall was sufficient to moisten the soil to a depth of at least 6 feet, and where the depth to

the underground water table was more than 15 feet, variation in depth of application or in frequency of irrigation did not affect the root distribution of the alfalfa." Furthermore, these investigators also found that "variations in number of irrigations, providing the same seasonal total was applied, did not materially affect the yields."

While Beckett and Huberty do not report soil-moisture contents, undoubtedly the upper layers of soil in the plots which received the more frequent applications had greater amounts of available water, for longer periods during the growing season, than those of the plots which were less frequently irrigated. The quantitative measurements of roots in 6 feet of soil, nevertheless, clearly show that their distribution in this depth of soil was not materially affected. It must be remembered that the soil in these plots was wet by rainfall to the full depth of 6 feet at the beginning of each season.

Weaver⁽⁸⁾⁽²⁰⁾⁽²¹⁾⁽²²⁾ and his co-workers have, however, reached different conclusions. They are of the opinion that changes in lateral spread of roots, depth of penetration, or output of branches are correlated in nearly every instance with changes in water content of the soil. These investigators further believe that by the application of more or less water, the root system may be varied, and yields will be affected.

Since it has been shown⁽¹⁴⁾ that drying of the soil below the surface layer is the result of root activity, and that dry soils must, then, necessarily mean the presence of roots, we believe that if the soil is wet to the full depth to which roots of the particular plant in question would normally go at the beginning of the growing season, then subsequent applications of water during the summer can have little influence on the extent of the distribution of the roots.

It is reasonable to assume that if soils are wet only to a certain depth, either by rainfall or by irrigation, and if the soil below this depth contains less moisture than that at which plants become permanently wilted, the roots will be confined within the moistened area. On the other hand, as far as the writers are aware, no experiments have shown that plants which normally are deep rooted can be made to keep their roots in the upper layers of soil, if those at lower depths have an available supply and if no other adverse conditions for root development are present in the lower strata. In other words, the progressive enlargement of the absorbing surface of the roots will cause them to elongate into the new moist layers, even though the region at present occupied by roots contains readily available water.

A number of references might be cited in which the effect of different soil-moisture conditions on the ratio of tops to roots of different plants has been studied. Most of this work has been with plants in containers, but in all cases, as has been previously⁽¹⁴⁾⁽¹⁸⁾ pointed out, serious objection can be raised to the conclusions drawn from studies on the water relations of plants, because of the inability to bring about or to maintain the predetermined moisture contents in the soil which the operators had presumed were maintained.

Studies on the water relations of deciduous fruit trees conducted at this station⁽¹⁴⁾⁽¹⁷⁾ have shown that apparently among the soils tested a *moist* soil has no optimum moisture content at which the trees grew best or at which the use of water was affected. Optimum moisture conditions for growth may therefore be taken to cover a range of soil moisture from the maximum field capacity to about the wilting coefficient. By analogy it might be reasoned that optimum conditions for root growth are covered within the same range as that found for the portion of the plant above ground, especially if a direct proportionality exists between top and roots.

It is certain that adverse conditions for root growth may be brought about by extreme variations in moisture conditions. Temperature and oxygen supply have been studied most in this connection. In the greater part of the work dealing with this subject, it is extremely difficult to gain an idea of the relative wetness of the soil under test, because there has been no uniformity in the manner of stating the soil-moisture conditions, and because no information is generally given to enable one to reduce the data to an understandable basis. There appears, however, to be in the literature on the subject of soil moisture and oxygen relations no conclusive evidence that favorable oxygen conditions for the growth of roots of agricultural plants would not be satisfied within the range of moisture contents from the moisture equivalent to about the wilting coefficient. The same statement may be made with regard to temperature conditions. Exceptionally frequent applications of water might, of course, result in filling the pore space of the soil for so long a time that unfavorable conditions for root growth would be established. The approximate equality of moisture equivalent to the maximum field capacity holds only for soils of fairly uniform texture throughout the depth considered. A decided change in texture (for instance, as Alway and McDole⁽¹⁾ have shown, a loam or clay above a sand) may result in the establishment, for some time after rain or irrigation, of a moisture content considerably higher

than the moisture equivalent in the same zone just above the coarse soil. This moisture content may sometimes be too great to permit root growth.

The curves in figure 3 and part of figure 2 suggest the probable history of the use of soil moisture by a single isolated row of sorghum. At the time of planting, we may assume that the soil to a depth of at least 6 feet was wet to the maximum field capacity, except that part composing the surface mulch. The developing seedlings send their roots into the soil, absorbing moisture as they go. Possibly because the roots near the crown are usually more abundant and have been in contact with the soil there for a longer time, more moisture has been absorbed from this region of the soil. The roots of the plants extending into the unplanted soil find no competition from roots coming from the opposite direction, as they would between adjacent rows in a normally planted field; in consequence, the moisture is absorbed progressively.

If, at some time during the growing season, the points of equal relative wetness in the soil, say 50 per cent, be joined, they will approximate part of the surface of a cylinder whose central axis would ideally (except for surface evaporation) coincide with the line of the border row. If a surface of relative wetness of 60 per cent were formed, it would be farther from the crowns of the plants, and roughly concentric to the other surface of 50 per cent; the same condition holds for surfaces of higher relative wetness. As growth takes place, more and more soil moisture is absorbed; and under conditions when there is usually a negligible amount of precipitation during the summer, this absorption will result in the gradual enlarging of all cylindrical surfaces of equal relative wetness. This process would be continued until the plants were matured or until the roots used up all of the readily available moisture in the depth of the soil possible of exploration by the roots of the plants growing there.

SUMMARY AND CONCLUSIONS

In all studies on the water relations of plants in which soil moisture is involved, much greater care should be given to the manner of taking the samples and to the interpretation of the results. The use of the moisture equivalent for the reduction of moisture contents to a common basis is suggested. Variations in soil texture that are not interpreted by moisture equivalents or by any other of the so-called soil-moisture constants may be great enough to make the results unreliable unless many samples are taken for each condition investigated.

The data in this paper indicate that moisture under rows of grain-sorghum plants is apparently extracted in successive zones and the extraction is progressive whenever no material additions of moisture occur during the growing season.

The percentages for relative wetness expressed as ratios of soil-moisture contents to their respective moisture equivalents, may be used to indicate the development of roots, and the results of adequate moisture samples, taken at proper times, indicate with a fair degree of accuracy the presence or absence of roots of plants growing on the soil tested.

A correlation has been shown to exist under the conditions of this study between the amount of roots and the extent to which the soil has been dried by root activity. The writers reason that if the soil is wet at the beginning of the growing season to the full depth to which roots of plants would normally penetrate, subsequent additions of water by rain or irrigation, unless adverse conditions for growth are brought about thereby, can have but little influence on the extent of the root system developed.

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THE MEDULLATED WOOL FIBER

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CLASSIFICATION OF HAIR MEDULLAE

The classification of the various types of medullae found among all types of hairs has been described by Hausman.⁽¹⁾ Medulla types of infra-hominid hairs are shown as (a) absent; (b) discontinuous, in which the medullary substance is displayed at fairly regular intervals throughout the length of the shaft; (c) intermediate, in which the medullary cells are found so closely contiguous as to present a medulla unbroken but uneven in contour; (d) continuous, in which the cells of the central lumen are apparently completely anastomosed or so closely packed as to give a regular tubular appearance to the fiber; and (e) fragmental, in which the medulla is found only as an occasional fragment at irregular intervals. Medulla types of human head hairs are classified as (a) absent; (b) fractional, which is seemingly almost identical with the fragmental type among infra-hominid hairs; (c) broken, in which the medulla may be heavy but does not form a continuous canal; and (d) continuous. Hausman has shown that among the infra-hominid hairs the greatest hair-shaft diameters were found in those carrying the fragmental medullae, while in human head hair, the largest were associated with presence of the continuous medulla.

The writer's examination of medullated wool fibers from several breeds of sheep, but largely from the Lincoln and Romney, indicates that the medulla types found in wool follow more closely Hausman's classification for human head hair than that for infra-hominid hair, the coarsest fibers being associated with the continuous rather than with the fragmental medulla.

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Blyth⁽²⁾ also classifies the medullae as continuous or discontinuous, depending upon whether or not the medulla persists throughout the length of the fiber or is interrupted at fairly long intervals. Fine medullae, much and irregularly interrupted, are called fragmentary, while those having a width of less than half that of the entire fiber shaft are termed rod-medullae. Practically all of the medulla types found by the writer among improved wools from the Lincoln and Romney breeds fall into the 'rod' classification.

The medulla in the wool fiber may be present to any degree between the fragmental and continuous classifications, along the entire length of the fiber, or along a portion of it only. The medullation may be at the proximal or distal extremities or at any point between them, although if present, it is usually found in the distal portion. Various types of medullae are often found in a single fiber. Occasionally a double medulla is to be observed (figs. 1, 2, 3).

DEFINITION OF MEDULLATED WOOL FIBER

The literature discloses the need for making some differentiation between the medullated wool fiber and 'kemp' or 'kemps.' Bliss⁽³⁾ points out that perhaps sufficient distinction is not drawn between true, short kemp and kempy or medullated wool.

The present paper makes no attempt to deal with true kemp as a medullated fiber, but is concerned rather with the intermediate type—the medullated wool fiber. The difference between the two kinds should be sharply defined, for kemp, in addition to being composed largely of the medulla, which is surrounded only by a thin wall of cortical and cuticular material, is usually shed in the fleece, whereas the medullated wool fiber is not. Kemp also has practically no tensile strength, is brittle and non-elastic, and presents a dead-white, opaque appearance. These characteristics are in sharp contrast to those of the medullated wool fiber.

THE SIGNIFICANCE OF THE MEDULLATED FIBER

Matthews⁽⁴⁾ describes the wool fiber as consisting of "several distinct portions: [including] (a) a cellular marrow, or medulla, which frequently contains more or less pigment matter . . ." He presents illustrations showing "wool fibers deficient in medullary cells," although he states in the text that the medulla is very frequently absent.

Woolman and McGowan⁽⁵⁾ speak of the medulla as if it were a normal constituent of the wool fiber and describe it as composed of cells, generally rounded in form. "The medullary canal may be broad and plainly visible; it may appear as a continuous or broken line, or it may not be seen at all in some fine, transparent wools. Granular fragments and often pigment matter may appear in the contents. Hair fibers commonly show the medulla, wool fibers often do not."

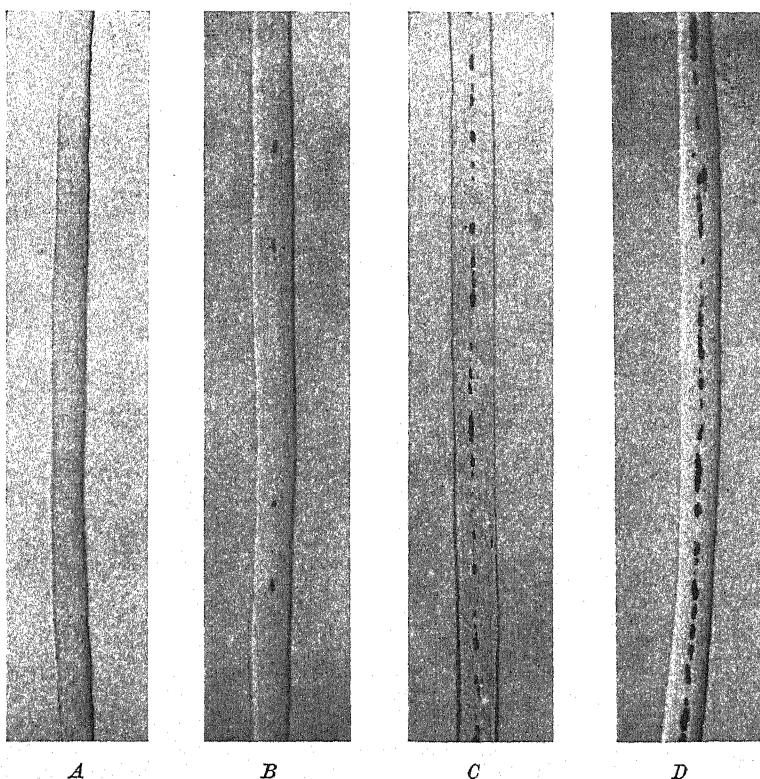


Fig. 1. Types of medullae found in wool ($\times 90$). *A*, absent; *B*, fragmental; *C*, fragmental; *D*, broken.

Hawkesworth⁽⁶⁾ considers absence of the medulla a defect, and states that inspection of the wool fiber reveals a central, or medullary part resembling marrow. To quote, "It has been noticed that in some fibers the central cells are wanting, and the fibers are, therefore, not perfect, which has much to do with many of the malformations we see in wool."

Bowman⁽⁷⁾ intimates that the medulla is perhaps a mark of differentiation between hair and wool and says that a wool fiber differs from a hair in having much less frequently a development of the central cells. The fallacy of differentiating between wool and hair on the basis of the presence or absence of the medulla is forcibly brought out by Lewis and Stöhr,⁽⁸⁾ who state that in man a medulla is lacking in many hairs, and that when present, in the thicker hairs, it does not extend their whole length.

Microscopic examination by the present writer of human head hairs, in balsam mounts, reveals that the medulla is often absent even in very coarse hair. Work at the California station indicates that in well-bred sheep of some of the long-wool breeds which are most subject to medullated fiber, the medulla is more often absent than present, although a great variation exists among individual animals within breeds.

Manufacturers of wool goods in England apparently have views at complete variance with those of Hawkesworth,⁽⁶⁾ and consider the medulla a serious defect. The matter was first brought to the attention of Romney sheep breeders in New Zealand by F. A. Aykroyd, a prominent English manufacturer, who suggested that New Zealand 'cross-breds' (wools of the medium and coarser grades not produced by the Merino) were deteriorating and at present left much to be desired from the manufacturer's viewpoint.† The criticism attracted the attention of breeders and elicited both favorable and unfavorable comment. In one of the trade journals⁽⁹⁾ a correspondent says in agreement with the criticism, ". . . I have frequently pointed out the absurdity of allowing sheep to live when they produce a mongrel fiber—a fiber that shows normal features for one-third of its growth from the base, while from that point to the tip of the staple, the fiber is only tubular." Answering a specific question put by the writer, Aldred F. Barker, Professor of Textile Industries in the University of Leeds, England, replied, "Medullation is considered to indicate a hair and consequently is anathema to the British manufacturer."

That the presence of the medulla affects the dyeing properties of wool has been indicated by King,⁽¹⁰⁾ who states that the presence of 'kempy' fibers in wool is of much concern to the trade, because the

† Mr. Aykroyd caused samples of wool to be prepared, illustrating his criticism. These samples consisted of small locks of Romney wool, from which a few "strong" or hairy fibers had been removed and mounted separately on the same background. The samples were sent to a New Zealand breeder of Romneys who in turn forwarded them to a Romney breeder in the United States. Through the latter, the writer was privileged to examine the samples. All of the so-called "strong" fibers to which Mr. Aykroyd took exception proved to be medullated.

inclusion of air gives them a different lustre and makes them appear to dye a lighter shade than normal fibers.

In a lecture before the Bradford Textile Industry, Sir James Parr,⁽¹¹⁾ High Commissioner for New Zealand, said, "During the past few years Bradford spinners have been insistent that our 44-48's wool is deteriorating, being characterized by too large a proportion of hair-like fibers and fibers which have a much greater diameter thickness at their tip than at their base, accompanied by reduction in luster and a lack of elasticity."

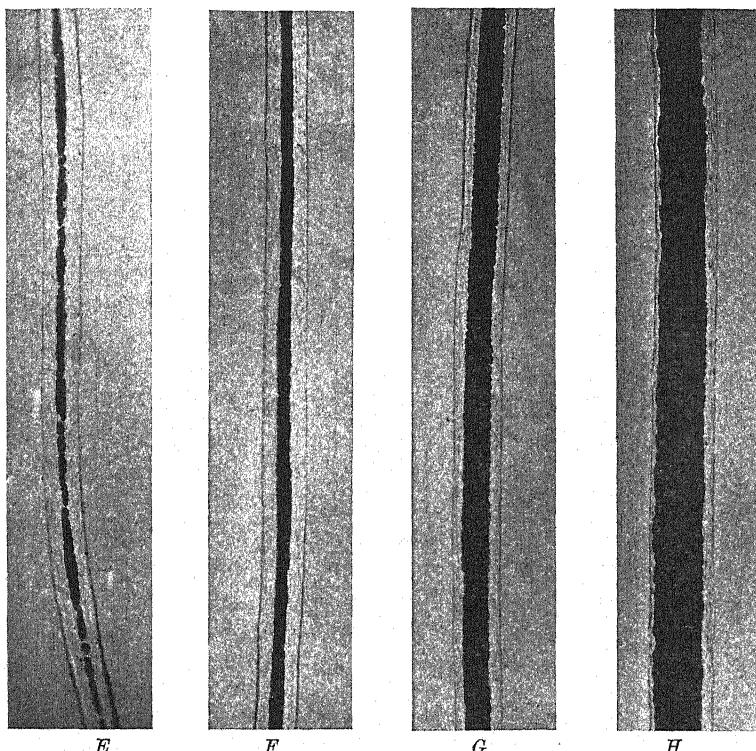


Fig. 2. Types of medullae found in wool ($\times 90$). E, broken; F, continuous; G, continuous; H, continuous.

CONTENT OF THE MEDULLA

Nathusius⁽¹²⁾ was the first to suggest that the medulla contained air. This view is now widely accepted. Hausman⁽¹³⁾ describes the medulla as being built up of many superimposed cells or chambers and containing air spaces and sometimes small masses of pigment material. Barker and King,⁽¹⁴⁾ measuring the fiber diameters and

medulla diameters of 120 fibers from the Scottish Blackface breed, found the medulla to occupy 50 to 60 per cent of the total fiber volume. They also found the medulla to be composed of about 90 per cent air space by volume, so that the actual medulla substance occupies only 10 per cent of the medulla core.

King⁽¹⁰⁾ in calculating the sulfur content of medullated fibers, by comparison with those which are non-medullated, states that the medullary material is practically devoid of sulfur, whereas the sulfur content of various wools is shown to range from 3.10 per cent in the Lincoln to 4.00 per cent in the Cape Merino.

ORIGIN OF THE MEDULLA

Duerden⁽¹⁵⁾ describes the medulla in kemp fibers as arising from a special group of cells located in the middle of the shaft and derived from the basal layer of the epidermis. In the case of the cuticle and cortex, keratinization is complete, and the cells become solid, while in the medulla the cell walls only are thickened with minute spinous projections, the cavity that remains in each cell filling with air as the cytoplasm disappears. He suggests that any fiber containing air should be classed as kemp, or kempy. Blyth⁽²⁾ states that in the formation of the medulla the cell walls always become keratinized and that the cell contents disappear before reaching the mouth of the follicle, possibly on account of a lack of nourishment caused by impermeability of the cell walls. King⁽¹⁰⁾ cites the fact that the medulla is sulfur-free, as being in consonance with the hypothesis that the medulla is an upward protrusion of the basal layer of the epidermis, which is almost sulfur-free. Thus the chemical aspects of medulla formation are at least compatible with the histological.

THE CAUSE OF THE FORMATION OF THE MEDULLA

King⁽¹⁰⁾ propounds an interesting speculative hypothesis relative to the cause of medulla formation. The fact that the medulla is sulfur-free is cited as an indication that it may possibly be caused by a diet deficient in sulfur. That the defect has been considered as extra-hereditary by others was shown by Sir James Parr: "In the past it has been thought that harshness, accompanied by hair and thickened tip, was rather a reflection of environmental than of genetic factors. . . . It is, however, being quite clearly shown that such is not the case. . . ."

Duerden⁽¹⁵⁾ in discussing the origin of kemp attributes the presence of 'kempy' fibers to the failure of the original outer protective coat of hair wholly to disappear in the process of evolution. He recalls the fact that in their natural state nearly all mammals have two types of hair, an outer coat of long, coarse fibers and an under coat of fine, short fibers. The presence of kempy fibers in the improved breeds is caused by the persistence of a portion of the outer or hairy coat.

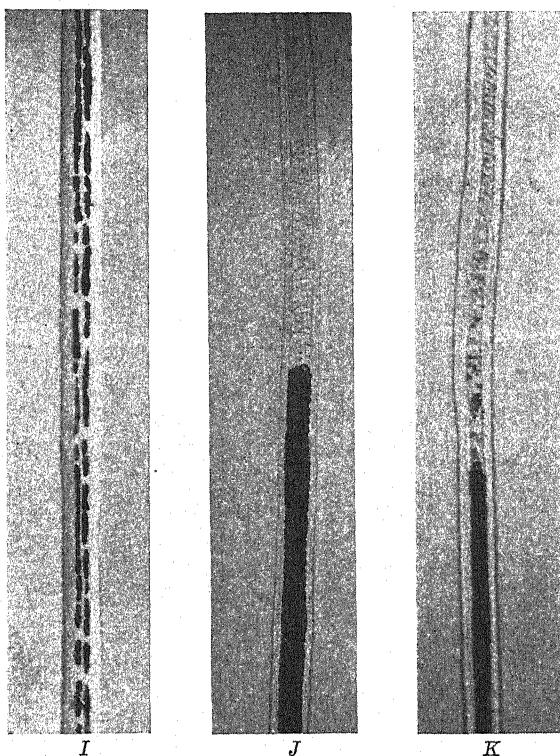


Fig. 3. Types of medullae found in wool ($\times 90$). *I*, double broken medulla; *J*, continuous medulla with air inclusions (black portion) and without air inclusions (light portion); *K*, continuous medulla showing, in the lower portion, complete air inclusions, and in the central portion, a few medullary cells containing air.

Confirmation of this theory is evidenced by the coat of the new-born Merino lamb, the entire body of which at birth is provided with long, straight, kempy hairs, each with a medulla and air inclusions. Such hairs are shed very early in the life of the lamb. Duerden evidently considers the problem of elimination of the medullated fibers as lying wholly outside the field of nutrition, for he states, ". . . . complete elimination should be possible by continued selection in breeding."

That nutrition is not involved in the formation of the medullated fiber has been partially verified by the writer. Sections through the epidermis of the thigh region were obtained from several Romney embryos taken late in gestation. The presence of medullated fibers was clearly shown, indicating that the medulla is formed before nutritional or environmental factors have had opportunity to become causative, unless the possibility of foetal malnutrition is given credence.

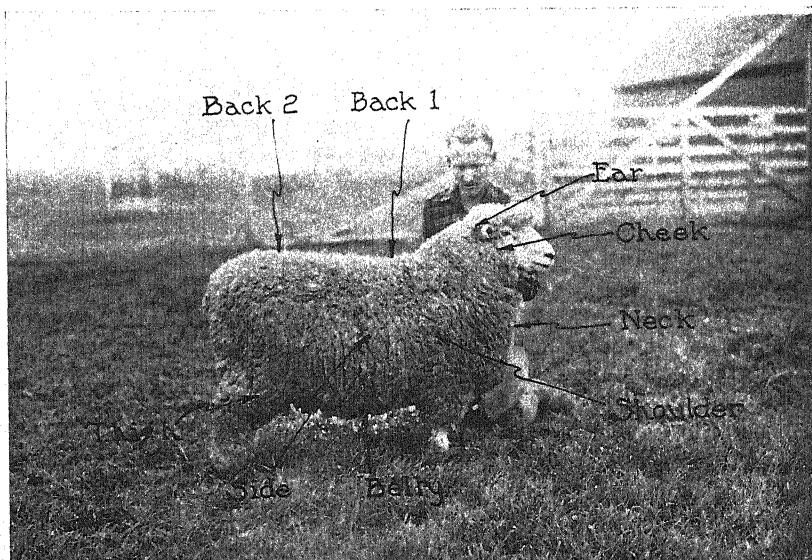


Fig. 4. The samples used in the study of the distribution of the medullated fiber within the fleece were obtained at the points indicated. (From *Hilgardia*, Vol. 3, No. 19.)

DISTRIBUTION OF MEDULLATED FIBERS

Hausman⁽¹³⁾ has shown that medullation is common in the hair covering of most animals. Duerden⁽¹⁵⁾ has found the defect in the Merino, which produces the finest wool known among improved breeds, and since a positive correlation exists between hair-shaft diameter and presence of the medulla (the coarser wools tending towards medullation) it seems logical to assume that medullation in the Merino fiber is indicative of its presence to some extent, at least, among all the breeds of sheep. Blyth⁽²⁾ has shown the presence of 'kemps' in four of the mountain long-wool breeds, six of the luster-wool breeds, and five of the Down breeds. Duerden and Spencer⁽¹⁶⁾ found medullated or heterotype fibers in the fleece of the Angora goat.

The writer,⁽¹⁷⁾ studying the distribution of medullated fibers in the fleeces of four Romney rams, found the forward portions of the fleece—the wool from the neck, shoulder, sides, and withers—to be much more nearly free from the defect than the rear portions from the thigh, the belly, and over the hips. There was, however, a great variation among the four individuals as to the proportions of medullated and non-medullated fibers and also as to the extent of the defect in certain parts of the fleece. Since this work was done, another test has been carried on with three purebred Romney wether lambs and with one registered Lincoln ewe. The lambs and ewe were kept together for a period of six months on a daily ration of 2.5 pounds of alfalfa hay and 0.75 of a pound of rolled barley. Thus any differences observed could hardly be attributed to the plane of nutrition unless there is considered the remote possibility of failure properly to metabolize the nutrients afforded. Samples were taken from each of the animals from nine different parts of the fleece (fig. 4). The fibers tested for medullation were taken at random by holding a staple and drawing from its side, one at a time, the 100 fibers nearest the right. Thus there was no tendency on the part of the operator to select the coarser fibers by virtue of their being more easily seen. No attempt was made to determine the sizes of the medullae microscopically or to classify them. Those fibers listed as "partly medullated" have discontinuous or fragmental types. The method of detection is described later.

The results in tabular form are given in table 1.

TABLE I
DISTRIBUTION OF MEDULLATED FIBERS IN VARIOUS PARTS OF THE FLEECE

Breed and name	Ear	Cheek	Neck	Shoulder	Side	Thigh	Back 1	Back 2	Belly	Sco-tum	Total
<i>Lincoln ewe 304*</i>											
Non-medullated.....	100	100	89	97	93	66	98	91	93	827
Partly medullated.....	0	0	11	3	7	28	2	9	7	67
Medullated.....	0	0	0	0	0	6	0	0	0	6
<i>Romney wether lamb 1147*</i>											
Non-medullated.....	100	100	70	77	67	51	100	87	92	100	844
Partly medullated.....	0	0	30	23	33	33	0	13	8	0	140
Medullated.....	0	0	0	0	0	16	0	0	0	0	16
<i>Romney wether lamb 1151*</i>											
Non-medullated.....	100	100	100	97	80	62	100	100	94	96	929
Partly medullated.....	0	0	0	3	20	30	0	0	6	4	63
Medullated.....	0	0	0	0	0	8	0	0	0	0	8
<i>Romney wether lamb 1152*</i>											
Non-medullated.....	100	100	100	100	100	100	100	100	100	100	1000
Partly medullated.....	0	0	0	0	0	0	0	0	0	0	0
Medullated.....	0	0	0	0	0	0	0	0	0	0	0

* Number refers to the University flock record.

The data presented in table 1 give partial support to the evidence obtained in the previous test. The largest proportion of defective fibers was found in thigh samples, although two of the present fleeces showed substantial numbers of partly medullated fibers on the forward portions.

The fact that wether lamb 1152 disclosed no medullated fiber is important. It proves that sheep of the long-wool types may produce fleeces without the defect.

BREAKING STRESS OF MEDULLATED AND NON-MEDULLATED FIBERS

A comparison of the breaking stress and extension at break of medullated and non-medullated Cotswold fibers was made by Speakman,⁽¹⁸⁾ who showed a mean percentage extension at break of 70.2 and a mean breaking stress of 1.51×10^6 grams per square centimeter for non-medullated fibers, while the corresponding figures for medullated fibers were 71.4 per cent and 1.20×10^6 grams per square centimeter, respectively. The variability in his results was high, but the data indicated that the medullated fibers were more elastic and less strong than the non-medullated ones. On the latter point he says, "From first principles, in view of the air spaces within the medullary cells of medullated fibers, it is to be expected that they will be weaker than non-medullated fibers. . . . Finally, the strength of medullated and non-medullated fibers is directly proportional to their cross-sectional area, although the former are weaker than the latter, as was to be expected."

A similar test of medullated and non-medullated fibers was conducted by the writer. Several hundred fibers of each kind were separated by the method later described, and then segregated into groups according to diameter at the midsection. All of the fibers used were taken from a single fleece of a Lincoln ewe. While no study was made of the various types of medullae present in the medullated group, it is certain that each would fall into either the "intermediate" or the "continuous" class described by Hausman.⁽¹⁾ No fibers showing the slightest defect were chosen for the non-medullated group, and no fibers failing to exhibit clearly the characteristic phenomenon presented by the truly medullated fiber were chosen. All of the "partly medullated" fibers were discarded.

The tests were carried on with a McKenzie fiber-testing machine. Atmospheric conditions were not controlled, although the testing of

the various groups was conducted in such a manner that fluctuations in humidity were impartial. The rate of loading likewise was not accurately controlled, but a serious attempt was made by the operator to turn at a uniform rate the screw device which applies the load in an apparatus of the type mentioned. Precautions were taken to insure a uniform tension on each fiber before it was secured between the clamps of the fiber-testing machine.

The data are here given in tabular form (table 2). The cross-sections of the fibers, exclusive of the cross-sections of the medullae, were not considered; the comparisons, therefore, are direct on the basis of outside diameters of the fibers at the midsection of each.

TABLE 2
BREAKING STRESS OF MEDULLATED AND NON-MEDULLATED WOOL FIBERS
FROM LINCOLN EWE
Distance between jaws of fiber tester (length of fiber), 2 inches

Midsection diameter of fiber Inches	Mean breaking stress			
	Medullated fibers Decigrams	Number of medullated fibers	Non-medullated fibers Decigrams	Number of non-medullated fibers
Under 0.0012				
.0012			231.50±26.72	96
.0013			267.39±29.53	112
.0014	380.78±46.37	14	301.75±32.30	106
.0015	357.29±39.66	21	349.04±36.61	104
.0016	415.93±47.69	58	344.10±43.23	123
.0017	428.24±35.50	62	409.77±44.97	130
.0018	465.15±62.81	46	426.99±39.37	67
.0019	501.13±63.71	48	455.79±46.18	34
.0020	506.07±59.60	88	492.00±28.53	23
.0021	507.19±58.99	43	581.67±36.65	9
.0022	532.12±59.77	74		
.0023	526.84±63.45	64		
.0024	560.49±62.49	55		
Over 0.0024	573.97±72.74	31		
Mean of fibers from 0.0014 to 0.0020 inch diameter, incl.	455.47		392.96	
Number of fibers from 0.0014 to 0.0020 inch diameter, incl.		337		490

The relative humidity of the room in which these tests were conducted varied from 33 per cent to 66 per cent during the course of the experiment.

These data seem to be at variance with those obtained by Speakman.⁽¹⁸⁾ While the probable errors are admittedly high, the fairly large numbers of fibers involved in each group tested, and the consistently higher breaking stress with each increase in fiber diameter, are indications of accuracy and point to the greater strength of the medullated fiber, regardless of the presence of the medulla.

In an attempt to prove the reliability of the results obtained, a number of 'partly medullated' fibers were broken in the apparatus. These fibers were carefully examined microscopically, and each was known to possess a discontinuous or broken medulla. Following the breaking of the fibers, the two fragments were again mounted and studied under the microscope. In each instance, it was found that the *break had occurred in the non-medullated portion of the fiber.* The results, therefore, support the data presented in table 2 and afford further evidence of the greater breaking stress of the medullated fiber.

EXTENSION AT BREAK OF MEDULLATED AND NON-MEDULLATED FIBERS

The observations on the extension at break of the same fibers studied in table 1 are recorded in table 3.

TABLE 3
EXTENSION AT BREAK OF MEDULLATED AND NON-MEDULLATED WOOL FIBERS
FROM LINCOLN EWE
Distance between jaws of fiber tester (length of fiber), 2 inches

Midsection diameter of fiber	Mean extensibility at break			
	Medullated fibers	Number of medullated fibers	Non-medullated fibers	Number of non-medullated fibers
<i>Inches*</i>	<i>Millimeters</i>		<i>Millimeters</i>	
Under 0.0012			26.47±1.85	96
.0012			25.88±2.15	112
.0013			27.08±1.98	106
.0014	23.57±4.62	14	26.78±1.75	104
.0015	25.67±2.52	21	26.34±1.72	123
.0016	23.29±3.12	58	25.30±1.83	130
.0017	21.05±3.63	62	25.61±1.45	67
.0018	22.30±4.87	46	26.62±1.40	34
.0019	24.06±4.02	48	26.83±1.08	23
.0020	24.07±3.65	88	27.44±1.28	9
.0021	22.98±3.90	43		
.0022	22.03±4.90	74		
.0023	22.44±3.72	64		
.0024	21.85±4.90	55		
Over 0.0024	18.80±4.78	31		
Mean of fibers from 0.0014 to 0.0020 inch diameter, incl.	23.22		26.12	
Number of fibers from 0.0014 to 0.0020 inch diameter, incl.		337		490

The humidity of the room in which these tests were conducted varied from 33 per cent to 66 per cent during the course of the experiment.

* The units are in inches because the machinist's micrometer used to measure the diameters of the fibers was constructed to read in inches. The McKenzie fiber-testing machine records the stretch in millimeters.

Here also the data are not in accord with those obtained by Speakman.⁽¹⁸⁾ The higher probable errors obtained with medullated fibers are perhaps due to disregarding the medulla diameters, and while these errors are sufficiently high to make inadvisable the drawing of any definite conclusions, again attention must be called to the consistency of the results and to the numbers of fibers tested.

THE EFFECT OF THE PRESENCE OF THE MEDULLATED FIBER UPON THE UNIFORMITY OF THE FLEECE

Hausman⁽¹⁾ has shown that the presence of the medulla is associated with increased hair-shaft diameter, both in infra-hominid and human head hairs. In the data given by Speakman,⁽¹⁸⁾ if it may be assumed that the fibers used were of common origin, the coarsest non-medullated fiber tested was practically of the same diameter as the finest medullated fiber. Blyth⁽²⁾ found in the heterotypic fibers that where the fiber diameter was greatest, the medulla was most likely to be found. She states, in the description of the mountain long-wools, that the medulla occurred more frequently in the coarse samples than in the fine. That the presence of the medulla tends toward the production of a coarse fiber is strikingly borne out by the fiber diameters given in table 2. It will be observed that no medullated fibers were found in the particular fleece under consideration, which were less than 0.0014 of an inch in diameter, whereas hundreds of non-medullated fibers of a diameter less than 0.0014 of an inch were readily available. Similarly no non-medullated fibers were found of a diameter greater than 0.0020 of an inch, while in the same sample medullated fibers up to 0.0024 of an inch were found in great numbers. Regardless, then, of whether or not medullation adversely affects the dyeing affinity, tensile strength, elasticity, softness, or any other attribute of the fleece, its presence is highly undesirable if it lends toward greater variability in the diameter of fiber. Until the medullated fiber is eliminated, it would seem impossible to produce the level, even fleeces so desired by the spinner.

THE DETECTION OF THE MEDULLATED FIBER

The medulla is easily detected under the microscope by preparing the fiber in a balsam or glycerin mount. Detection is sometimes difficult in a water mount and is often impossible when the fiber is mounted dry.

Realization of the desirability of having a macroscopic method for accurate detection of the medulla led the writer to the discovery of a method described in a previous paper.⁽¹⁹⁾ Since that time, however, certain changes in technique have been adopted, and a new description, although necessitating partial repetition, seems warranted.

A square or rectangular piece of glass about 5 inches by 8 inches (a discarded photographic plate is excellent) is placed horizontally over a black or dark blue background. Melted paraffin is then slowly poured on the glass in such a manner as to form a wall about one-quarter to three-eighths of an inch high and in the form of a pair of parentheses with ends completely joined. At the top of the 'parentheses' the wall of paraffin is of full height, but at the bottom, which is placed almost at the lower edge of the glass plate, the wall is depressed to a height of about one-eighth of an inch. The 'parentheses' should be about six inches in the longest dimension and about three inches across at the point of greatest width.

Into the area surrounded by the paraffin wall a quantity of clear glycerin sufficient to fill the 'parentheses' is poured.

Fibers are examined in a room lighted only by a dim red light, such as is used for photographic dark rooms. This light is suspended about twelve inches above the shallow lake of glycerin and sufficiently far forward to allow the operator to make close observation of the fibers without coming in contact with the lamp. The sample to be tested is cleaned in benzene, and fibers are subjected to treatment one at a time.

The distal end of the fiber is held with a pair of fine tweezers, the proximal end with the fingers. The fiber is then submerged in the glycerin, the end held between the fingers being allowed to pass over the paraffin wall at the lower end of the 'parentheses,' the end nearest the operator. For examination of every part of the fiber, the ends may be reversed.

If the fiber is non-medullated, it cannot usually be seen with the naked eye when submerged in the glycerin under the subdued light. Very coarse non-medullated fibers, such as are to be found in the Lincoln and Cotswold breeds, can sometimes be seen, but their appearance offers slight contrast with that of the glycerin. If the fibers are medullated, the medulla, if it contains air inclusions, persists as a fine, white line which can readily be seen.

In some fibers, a clearly defined medullary substance, which exhibits no air inclusions, may be found. These fibers, which as yet have been observed in only one fleece, are not detectable by the method

described. It is probable, however, that they are so few in number as not seriously to affect the efficiency of the method.

Tests of the efficacy of this method of detection have shown that it is quite satisfactory. At first the operator should check results with the microscope, but experience has shown that proficiency in detection may be acquired to a point where subsequent proof is superfluous. A fiber with intermittent or fragmentary medulla is easily detected, the medullated areas being revealed as tiny white spots. Accuracy in detection seems, after reasonable practice, to be regulated only by the eyesight of the operator.

For less exacting work, the operation may be carried on in natural light, subdued to such a point that reading would be difficult. The advantage of the artificial light apparently lies only in its uniform intensity.

THE ELIMINATION OF THE MEDULLATED FIBER

While it is conceivable that factors extraneous to heredity are partially responsible for the formation of the medulla, the evidence thus far points strongly to the field of genetics. If, then, it is a genetic problem, the elimination of the medullated fiber lies wholly in the hands of the breeder. If the problem is to be solved the breeder should adopt a test for the presence of medullated fibers in the fleeces of stud animals, especially in the fleeces of stud rams. The results of such a test should be used as a partial index of the desirability of the animal for breeding purposes.

The glycerin test herein described affords an ideal method for the husbandman's use. It requires no technical training and no expensive equipment. Furthermore, it yields the information needed by the breeder—whether or not the fiber is medullated and contains air inclusions.

The results of the study on the distribution of the medullated fiber within the fleece, presented in table 1, indicate that it is possible to produce sheep of the long-wool breeds which carry fleeces without the defect.

SUMMARY

A sharp differentiation should be made between the medullated fiber and kemp.

The medullated wool fiber is thought to be responsible for serious difficulties encountered in wool manufacturing.

The medullated fiber is found among many kinds of animals, probably among all of the breeds of sheep, and is common among those breeds producing the coarser grades of wool. It is likely to be found in any part of the fleece, but is most apt to occur in the rear portions notably on the lower thigh. Some sheep are entirely free from the defect.

Breaking stress data reported in this paper show medullated fibers to be stronger than non-medullated fibers of the same diameter at the midsection.

Tests on the extension at break indicate that non-medullated fibers may be more elastic than medullated fibers of equivalent midsection diameter.

The medullated fiber is partially responsible for wide deviations from the mean diameter of fiber of the fleeces in which it occurs.

The medullated fiber may be detected microscopically by the use of several mounting media. It may be detected accurately by a macroscopic method described in the text. The macroscopic method of detection is simple and inexpensive, and may be used, with practice, by the breeder.

The elimination of the defect is probably within the field of genetics. The fact that some animals of the long-wool breeds are free from the defect, indicates that the problem of elimination is possible of solution.

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THE EFFECT OF DORMANT PRUNING ON THE CARBOHYDRATE METABOLISM OF VITIS VINIFERA

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In previous papers^(2, 3, 4) experiments were described, the results of which showed: (1) that dormant² pruning depresses the capacity of the vines for total growth; (2) that dormant pruning depresses the capacity of the vines for fruiting; (3) that dormant pruning reduces the quality of the fruit of some varieties; and (4) that bearing depresses the capacity of the vines for growth.

The purpose of this paper is to describe the effect of dormant pruning on the carbohydrate metabolism of the vine and to discuss the influence of this effect on growth and fruiting.

TYPES OF PRUNING USED

The plan of the plantings and the various types of pruning employed were those described in detail in the earlier papers.^(2, 3) The types of pruning used on the vines from which samples for analysis were taken were:

Non-pruned, part crop: no pruning. All clusters in excess of what was thought necessary for a good crop of fruit were removed before blooming.

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² The depressing effect of herbaceous or summer pruning on growth and crop is generally recognized by viticulturists; see: Bioletti, F. T., and F. C. H. Flossfeder. Topping and pinching vines. California Agr. Exp. Sta. Bul. 296:369-384. 1918.

Cane-pruned, part crop: pruning similar to that commonly used in California on Sultanina (Thompson Seedless) except that about 25 per cent more wood was retained. All clusters in excess of what was thought necessary for a good crop of fruit were removed before blooming.

Normally (spur) pruned: pruning as nearly as possible that of the best accepted commercial practice with the varieties used. All clusters allowed to develop.

Severely pruned: pruning similar to that of the normally pruned vines but more severe, only the base buds being retained on the spurs. All clusters allowed to develop.

METHODS OF SAMPLING AND ANALYSIS

The woody material for analysis was collected as follows:—

The Shoots.—Two entire shoots were taken from each vine. About four inches of the basal portion of each shoot was taken for a sample. All the basal portions of the shoots from a single pruning treatment for a given date were thrown together to make one sample.

The Trunk.—Disks 3 to 6 millimeters in thickness were sawed from the similar positions in the trunks of the vines as they were being removed. Two vines were usually removed from a given pruning treatment, and in such cases the sample was made up of wood of both vines.

The roots.—Segments of the main roots at a distance of twenty to twenty-five centimeters from the root crown were collected at the time vines were being removed. Here also a single sample was made up of wood from two vines.

Preparation of the Samples.—In all the samples, the bark was separated from the wood. The material was then cut into small segments of less than one cubic centimeter in size and placed immediately in an oven with forced ventilation at 65° C. The dried material was ground so that it would pass through a 60-mesh sieve.

Analysis.—³For the determination of reducing substances the extraction was made with 95-per cent alcohol, while the starch was hydrolyzed with taka-diastase. The reductions were carried out according to the Shaffer-Hartman method.⁽¹⁾ Duplicate determinations were always made.

³ The writer is indebted to Miss Isabelle D. Collins and Mr. T. C. Broyer, technicians in the Division of Viticulture, who made the chemical analyses.

THE EFFECT OF PRUNING ON THE CARBOHYDRATES OF THE VINE

Some ways in which pruning affects the vines, as shown in previous papers, are to depress the growth and to decrease the bearing and with certain varieties, to lower the quality of the fruit. The effect on the quality of the fruit is especially pronounced in the Muscat of Alexandria. The greater vine growth of the lightly pruned and non-pruned vines may be a result of more favorable nutrition of the vine over the entire season. The improvement in fruiting, on the other hand, must be due to more favorable nutrition when the individual flowers are developing and the berries setting; that is, before blooming, during blooming, and during the setting of the berries.

In the vine, the later stages in the development and opening of the flowers do not take place before and during leafing out in spring as they do with most deciduous fruits. By the time of leafing out in spring, the development of the vine flower has proceeded to the primordia of the individual flowers, but the development of the flower parts, such as anthers, style, and ovules, occurs while the vine is in leaf, and the flowers open six to eight weeks after the beginning of active growth. It seems probable, therefore, that the effects on fruiting of changes in carbohydrate nutrition could be produced only during early summer, that is, previous to the middle or end of June at Davis.

With the above facts concerning the fruiting habit of the vine, together with its responses to pruning, in mind, analytical results obtained in a study of the effects of pruning on the carbohydrate nutrition of the vine at Davis will be presented.

The Seasonal Changes in Total Carbohydrates.—During the season of 1925 series of samples of the vine parts to be analyzed were collected from the Muscat of Alexandria from January until well along in the growing season. A similar series of samples was collected from the Monukka in 1926. Owing to the similarity of the results obtained with the two varieties, only the results for the Muscat are shown in figure 1. This similarity of results for the two varieties during early summer may be illustrated by a comparison of the graphs of figure 1 with those of figure 3A. Also, since the effect of pruning on the seasonal change of carbohydrates in the vine parts analyzed was confined almost entirely (fig. 3) to the shoot, results for this part of the vine alone are shown in figure 1.

The graphs of figure 1 indicate two maxima and two minima in the seasonal changes in the total carbohydrates for the non-pruned,

cane-pruned, and normally pruned vines, one maximum falling in the dormant season and the other during the early part of the growing season. This, no doubt, is the normal condition for the seasonal behavior in the carbohydrates of the vine at Davis. The severely pruned vines, on the other hand, fail to show a maximum in the early part of the growing season.

Pruning affected the amount of total carbohydrates present at both the maximum of the dormant season and that of the early part of the growing season. The effect was much greater at the vegetative

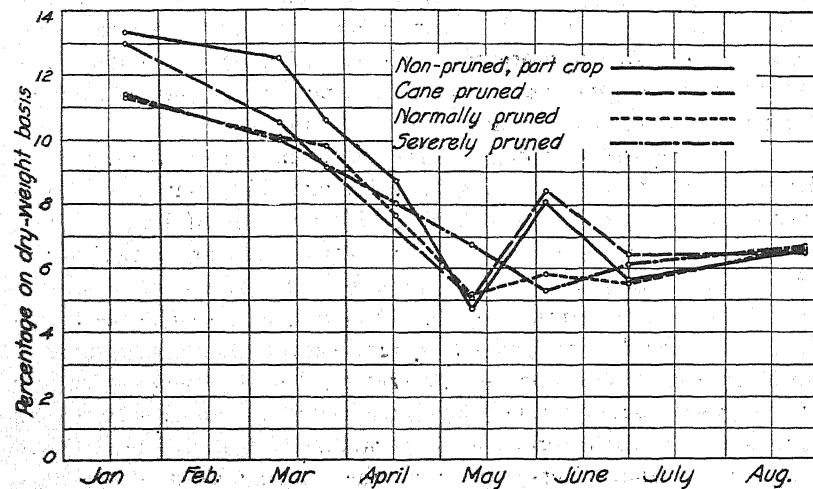


Fig. 1. The effect of pruning on the percentage of total carbohydrates in the basal portion of Muscat of Alexandria canes and shoots at various dates during 1925.

maximum. At this time the amount of total carbohydrates in the non-pruned vines was 36 per cent greater than in the normally pruned vines and 40 per cent greater than in the severely pruned vines; that in the cane-pruned vines was 44 per cent greater than in the normally pruned vines and 49 per cent greater than in the severely pruned vines. The amount of total carbohydrates in the non-pruned and cane-pruned vines at the dormant maximum was 18 and 15 per cent respectively greater than that of the normally pruned vines. At this season there was no difference in the carbohydrate content of the normally and severely pruned vines.

In the periods between the two maxima, no significant influence of pruning on the amounts of carbohydrates present is indicated. This lack of noticeable influence could probably be accounted for by differences in the rates of growth. The non-pruned vines start growth

earliest, and, owing to the many shoots that develop, the rate of growth rapidly diminishes. The more severely pruned vines, on the contrary, start growth later, and, owing to the relatively few shoots that develop, the rate of growth is rapid and continues to be so over a relatively long period. This continued rapid length growth of the severely pruned vines, no doubt, prevents the appearance of a maximum in the total carbohydrates during early summer, these materials being consumed as rapidly as they are produced.

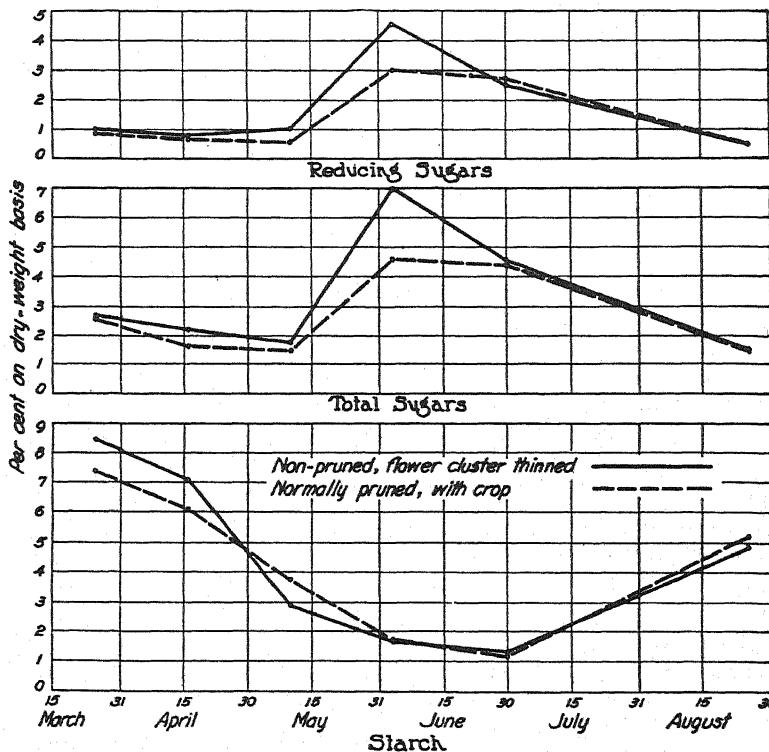


Fig. 2. The effect of pruning on the percentage of reducing and total sugars and starch in the basal portion of Muscat of Alexandria shoots.

The Amounts of Sugars and Starch Present.—By plotting graphs for each of the carbohydrates determined—namely, reducing sugars, total sugars, and starch—as in figure 2, the effect of pruning upon the changes in each of these substances is indicated. For these graphs the figures for the basal portion of the Muscat shoots are again used. Similar figures for the reducing sugars in the Monukka are shown in figure 3 A.

The graphs of figure 2 show that the influence of pruning, especially during early summer, was primarily, if not solely, confined to the carbohydrates in solution—sugars. There was 52 per cent more reducing sugars in the non-pruned than in the normally pruned vines on June 3, and 51 per cent more total sugars.

For starch, on the contrary, there is no indication that pruning affects the amount present during early summer. There is, however, a higher percentage of starch present in the non-pruned than in the normally pruned vines during the dormant season. This larger amount is, no doubt, of importance in connection with the greater capacity of the non-pruned vine, yet it is of questionable significance with regard to the better setting and development of the fruit which some varieties exhibit with cane-pruning or non-pruning accompanied by flower-cluster thinning. The improvement in fruiting is more closely associated with the larger amounts of the carbohydrates in solution. This association will be indicated more clearly in the following sections of this paper.

The Amounts of Sugars in the Different Parts of the Vines.—As stated, samples for analysis were collected from the shoots, trunk, and roots. The effect of pruning on the sugars in these parts of the vine during early summer is indicated by the graphs of figures 3, which represent results obtained with Monukka. As shown in figure 2, the starch content of the vine was not influenced in early summer by pruning. Also, since the direction and magnitude of the effect of pruning on the reducing and total sugars was very similar (fig. 2), only the data for the reducing sugars are included.

The graphs of figure 3C indicate that pruning has little or no effect on the amounts of reducing sugars present in the roots during early summer. The differences which are indicated seem to be due to differences in the time of the beginning of growth and the rates at which this substance is used for growth rather than to the type of pruning.

As indicated by the graphs of figure 3B, there was even less difference in the amounts of reducing sugars present in the trunks of the vines under the several types of pruning than in the roots.

In the basal portion of the shoots there was an indication of a marked influence of pruning on the amounts of sugars present. The amount of reducing sugar in this part of the non-pruned Monukka vines was slightly greater than that indicated for Muscat of Alexandria in figure 2. The amount was 33 per cent larger than that in the normally pruned vines of this variety on May 19. The graph of figure 3A also indicates that the changes in reducing sugars during

early summer in the Monukka follow a course very similar to that for the total carbohydrates in Muscat of Alexandria, shown in figure 1. For the Monukka there was no summer maximum in reducing sugar with severe pruning, a condition which is in agreement with that found for Muscat.

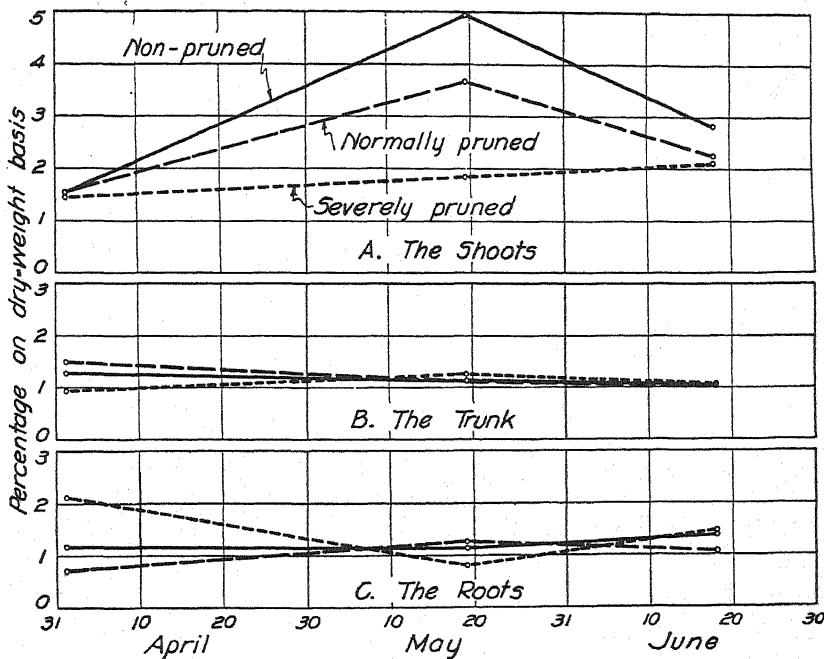


Fig. 3. The effect of pruning on the percentage of reducing sugar at various levels in the Monukka vines during early summer.

THE EFFECT OF PRUNING ON THE RATE OF GROWTH OF THE VINE

The fact noted that larger amounts of certain of the carbohydrates are present in cane-pruned and non-pruned vines than in the normally and severely pruned vines, especially during early summer, makes it of interest to inquire further into the causes of these differences in nutrition. Does the greater reserve of the dormant cane-pruned and non-pruned vines carry over to give these vines a more favorable nutrition during early summer, or does the pruning affect the rate or type of growth so as to favor the production and accumulation of sugars?

The Amounts of Reducing Sugars and Total Carbohydrates Present in the pruned Vine.—The removal of several series of vines made it possible to obtain weights of the vines both before and after pruning. From these figures, together with the analytical data, it was possible to estimate the amounts of carbohydrates contained in the vines after pruning. The average weight of vine after pruning and the percentage and total amount of reducing sugars and total carbohydrates present in two five-year-old Monukka vines under severe, normal, and non-pruning, are shown in table 1.

TABLE 1

THE WEIGHT OF DORMANT VINE AFTER PRUNING AND THE AMOUNTS OF REDUCING SUGARS AND TOTAL CARBOHYDRATES TO A VINE BASED ON DRY WEIGHT

Average of two vines

Measurements	Non-pruned	Normally pruned	Severely pruned
Weight of vine after pruning, kilos.....	39.10	12.70	4.50
Per cent of reducing sugars.....	1.25	1.16	1.13
Per cent of total carbohydrates.....	7.10	6.48	6.22
Weight of reducing sugars per vine, kilos.....	0.49	0.14	0.05
Weight of total carbohydrates per vine, kilos.....	2.78	0.82	0.28

Although the differences between the percentages of reducing sugars and of total carbohydrates in the non-pruned vines and those in the severely and normally pruned vines during dormancy, are relatively small, the total amounts of these substances retained in the non-pruned vines after pruning is much larger than that of the severely and normally pruned vines. They contained almost four times as much of each of these substances as the normally pruned and ten times as much as the severely pruned vines.

The larger amounts of these carbohydrate materials present in the non-pruned vines, no doubt, endows them with a greater potential capacity for growth and fruiting, yet if the rate and mass of growth produced by the vines under the several types of pruning was in proportion to the amount of wood retained at pruning, this difference in the amounts of the reserves during dormancy would not account for the differences in nutrition that exists during early summer.

The Rate of Growth.—During two seasons when Monukka vines were being removed at different intervals from May to July, weighings were made of the several parts of the vine, such as leaves, shoots without leaves, and trunk. The weights of the leaves and shoots were obtained in as short a time as possible to avoid error due to the loss of moisture. From these weighings it was possible to determine the

ratio of weight of shoots produced during early summer to total weight of the parts of the vine retained at pruning, and the ratio of weight of leaves to weight of shoots for vines of each of the types of pruning. These ratios for the non-pruned, normally pruned, and severely pruned vines are shown in figure 4.

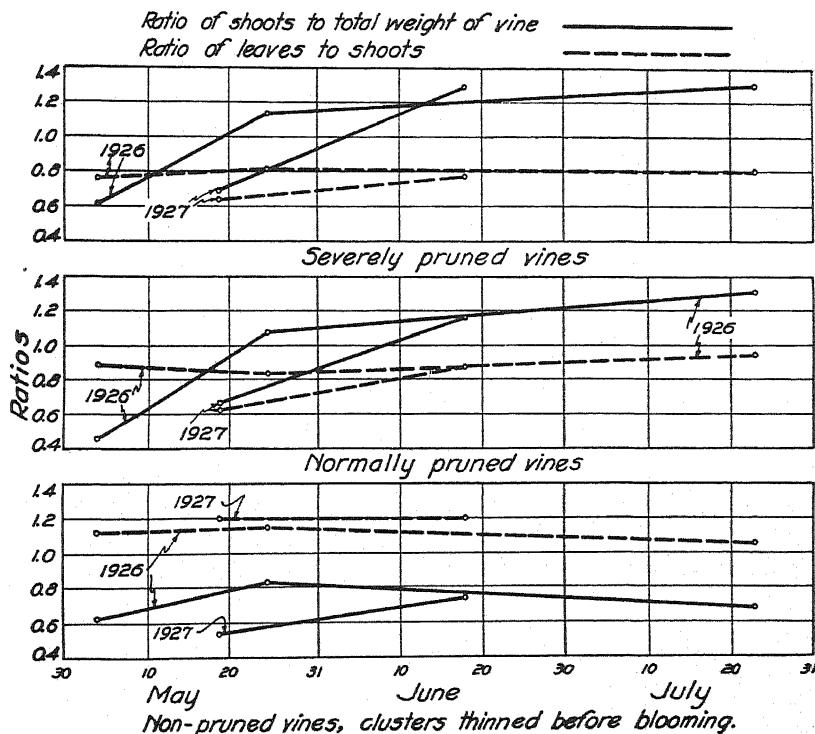


Fig. 4. The graphs for the severely pruned vines show that in 1926 the ratio of weight of shoot to total weight of the parts of the vine retained at pruning was 1.13:1 on May 25. This ratio continued to increase until on July 23 it was 1.30:1. At both dates the ratio of weight of leaves to weight of shoot was only 0.80:1. A similar condition obtained in 1927. In this season the ratio of weight of shoots to total weight of vine did not become greater than 1 so early, however, and the ratio of leaves to shoots was smaller.

The graphs for the normally pruned vines show that in 1926 the ratio of weight of shoot to total weight of the parts of the vine retained at pruning was 1.13:1 on May 25. This ratio continued to increase until on July 23 it was 1.30:1. At both dates the ratio of weight of leaves to weight of shoot was only 0.80:1. A similar condition obtained in 1927. In this season the ratio of weight of shoots to total weight of vine did not become greater than 1 so early, however, and the ratio of leaves to shoots was smaller.

In the case of the normally pruned vines, the type of growth was very similar to that of the severely pruned. The ratio of weight of

shoots to total weight of the parts of the vines retained at pruning was 1.07:1 on May 25 and 1.30:1 on July 23. For this type of pruning the ratio of leaves to shoots was slightly higher than that of the severely pruned vines, being 0.83:1 on May 25 and 0.94:1 on July 23. The ratios for the normally pruned vines during 1927 bear the same relation to those of the severely pruned vines as that just indicated for the year 1926.

In the non-pruned vines there is a complete reversal of the conditions just indicated for the severely and normally pruned vines. With this type of pruning the ratio of weight of shoots to total weight of the parts of the vine retained at pruning never attained 1. On May 25 it was 0.84:1 and on July 23 0.70:1. On the other hand, the ratio of weight of leaves to weight of shoots was greater than 1 at the earliest weighing on May 4 and remained so. Again in 1927 the ratio of weight of shoot to total weight of vine at the beginning of the growing season ranges from 0.56:1 on May 19 to 0.74:1 on June 18. On these same dates the ratio of weight of leaves to weight of shoots was 1.20:1.

The graphs of figure 4 thus show that the non-pruned vines are making *less shoot growth* in proportion to the weight of vine at the beginning of the growing season than either the normally or severely pruned vines. On the other hand, these same non-pruned vines are producing a *much greater weight of leaves* in proportion to shoots than the normally or severely pruned vines.

Rate of Total Shoot Elongation and Weight of Leaves.—Although the ratio of weight of shoots to total weight of the parts of the vine retained at pruning in the non-pruned vines was much less than that in either the severely or normally pruned vines, a condition which would tend to conserve the reserve materials of the vines, the rate of total shoot elongation under this type of pruning was much greater than that of the pruned vines owing to the larger number of shoots. The effect of pruning on the time and amount of total length growth of all shoots to a vine is shown in figure 5.

The graphs of figure 5 indicate that the type of pruning modifies both the rate of growth and the amount of total length growth. The importance of this effect is seen when the amount of length growth found at the several dates of measurement is taken into account. On May 13 the non-pruned vines had made 74 per cent of the total season's length growth, while the normally and severely pruned vines had made only 23 and 12 per cent respectively. Also, by May 13 the non-pruned vines had made 90 and 95 per cent respectively as much

length growth as the normally and severely pruned vines made during the entire season.

The figures indicate a very rapid total elongation of the shoots for the non-pruned vines during April and early May. After this, the rate of growth moderates so that very little additional elongation of the shoots occurs between July 1 and the end of the growing season. On the contrary, the rate of total elongation of the shoots of the

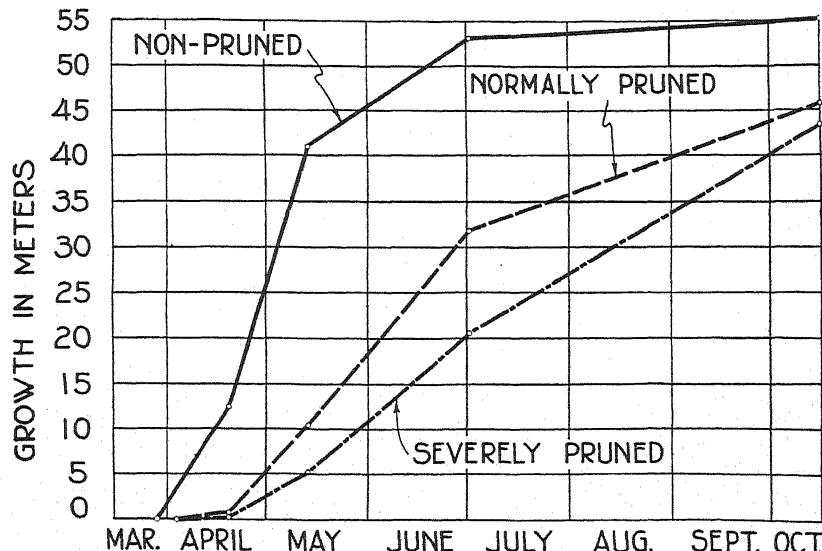


Fig. 5. The effect of pruning on the start, rate, and total length of cane growth.

severely pruned vines is very slow in April and early May. It is very rapid during the latter part of May and June and continues so with but little moderation until the end of the growing season. The growth curve for the normally pruned vines is intermediate between those of the non-pruned and those of the severely pruned vines. Yet its contour is very similar to that of the severely pruned vines.

Not only was the rate of total shoot growth greater in the non-pruned vines than in the normally pruned vines, as indicated in figure 5, but the total weight of leaves to a vine was much larger, especially during the early summer. The effect of type of pruning on the weight of leaves to a vine is shown in table 2.

The figures of table 2 show that on May 19 the non-pruned Monukka vines had produced almost five times as great a weight of leaves as the severely pruned and three times as great a weight of

TABLE 2

THE EFFECT OF TYPE OF PRUNING ON THE WEIGHT OF LEAVES TO A VINE

Variety	Date of weighings	Type of pruning and weight of leaves, in kilos		
		Severe	Normal	Non-pruned
Black Monukka.....	May 19, 1927.....	1.05	2.10	7.10
	June 18, 1927.....	2.70	4.50	11.60
	May 5, 1925.....	0.06	0.18	3.87
Muscat of Alexandria.....	June 24, 1925.....	0.85	1.54	5.97
	Oct. 15, 1925.....	3.06	3.47	8.80

leaves as the normally pruned vines. On June 18 the ratios of weight of leaves of the non-pruned to that of the severely and normally pruned vines was 4.30:1 and 2.58:1 respectively. The greater weight of leaves in favor of the non-pruned Muscat of Alexandria vines during May and June was even larger than that for the Monukka. The figures for the Muscat of Alexandria further show that the great advantage in total weight of leaves to a vine in favor of the non-pruned vines obtains throughout the growing season; there being two and one-half times as great a weight of leaves on the non-pruned as on the normally pruned vines, and three times as great a weight of leaves on the non-pruned as on the severely pruned vines on October 15.

The rate of growth and the total weight of leaves produced, as shown in figures 4 and 5 and table 2, clearly show that the non-pruned vines have a great advantage over the normally and severely pruned vines for the manufacture and accumulation of carbohydrates. The non-pruned vines are utilizing a smaller proportion of the available materials for shoot elongation at all stages of development, but especially during early summer, than either the normally or severely pruned vines. Then again, at all times during the growing season they have a much greater actual weight of leaves than the pruned vines, for the production of carbohydrates.

The differences in rate of growth which very markedly alter the production of leaves under the different types of pruning seem to be quite sufficient to account for the differences in the carbohydrate nutrition as indicated in figures 1, 2, and 3. These differences in rates of growth during early summer endow the non-pruned vines with a weight of leaves several times greater than that of either the normally or severely pruned vines. This is equivalent to a considerable increase in the season of leaf activity for the non-pruned vines. At the same time the growth of the non-pruned vines is exerting a smaller drain on the reserves of the dormant vines than

the less favorable growth made by the pruned vines, which, no doubt, accounts for the much higher maximum attained in the amounts of carbohydrates present in the shoots of these vines during May and June (figs. 1 and 2).

THE RELATION OF RATE OF GROWTH AND CARBOHYDRATE NUTRITION TO FRUITING

In the foregoing sections of this paper the effects of pruning on several of the carbohydrate materials and total carbohydrates present in the vine parts have been shown. It has been shown also that the effect of pruning on the rate of growth, and still more on leaf production, apparently accounts for the changes in carbohydrate nutrition. The relation of these changes in growth and nutrition to the fruiting of certain varieties of vines will now be indicated.

The Effect of Carbohydrate Nutrition on Flower Development.—In an attempt to correlate the number of leaves to a vine with the carbohydrate nutrition of its flowers and to determine the influence of this carbohydrate nutrition on flower development, the number of leaves to a vine was altered. To increase the number of leaves to a vine above that of the normally pruned vines, cane-pruning with flower-cluster removals to control the crop was employed. The excess clusters were removed about four weeks before blooming. To reduce the number of leaves to a vine below that of the normally pruned vines, defoliation was employed. All the leaves on ten normally pruned vines of Muscat of Alexandria and Monukka and four vines of Alicante Bouschet were removed two weeks before blooming. All clusters on the normally pruned vines were allowed to develop to maturity, since with this treatment crop is sufficiently controlled by the winter pruning.

At the time the first flowers on the clusters opened, samples⁴ for analysis were collected. The average results for triplicate samples for reducing and total sugars, together with the percentage germination of pollen in 15 and 20 per cent sucrose media are given in table 3.

The figures of table 3 indicate a positive correlation between the number of leaves to a vine and the sugar content of the mature flowers. In the Muscat of Alexandria the amounts of reducing sugars

⁴ Parts of fifteen to twenty clusters were put together for a single sample, which was weighed immediately and then plunged into boiling alcohol to which a bit of calcium carbonate had been added. The extractions and reduction were carried out the same as with the wood samples.

TABLE 3

THE EFFECT OF NUMBER OF LEAVES TO A VINE ON THE PERCENTAGE OF SUGAR
PRESENT IN THE OPENING FLOWERS AND THE GERMINABILITY OF POLLEN
IN MUSCAT OF ALEXANDRIA, ALICANTE BOUSCHET, AND MONUKKA

Treatment	Number of leaves to a vine		Reducing sugars, per cent*		Total sugars, per cent*		Germination of pollen, per cent	
	1927	1928	1927	1928	1927	1928	1927	1928
MUSCAT OF ALEXANDRIA								
Cane-pruned.....	1,700	1,950	0.80	0.97	1.50	1.67	37.0	38.0
Normally pruned.....	760	886	.74	.86	1.33	1.43	12.0	11.0
Defoliated.....	0	0	.67	.48	1.09	0.91	3.0	4.0
ALICANTE BOUSCHET								
Cane-pruned.....	1,650	‡	.60	.93	1.08	1.61	43.0	38.0
Normally pruned.....	870	‡	.46	.73	0.88	1.42	12.0	9.0
Defoliated.....	0	‡	0.38	0.39	0.82	0.64	1.0	3.0
MONUKKA								
Cane-pruned.....	3,800	0.61	1.14	46.0
Normally pruned.....	1,390	0.60	1.08	16.0
Defoliated.....	0	0.38	0.67	5.0

* Per cent of sugars on basis of the fresh weight.

‡ Leaves not counted.

present in the flowers of the normally pruned vines with leaves was 10 per cent larger than in the defoliated vines in 1927 and 80 per cent larger in 1928. The amount of total sugars in the flowers of the normally pruned vines with leaves was 22 per cent larger than in the defoliated vines in 1927 and 56 per cent larger in 1928. The amount of reducing sugars in the flowers of the cane-pruned vines was 19 per cent larger than in the defoliated vines in 1927, and 93 per cent larger in 1928; the amount of total sugars in the flowers of the cane-pruned vines was 38 per cent larger than in the defoliated vines in 1927 and 84 per cent larger in 1928. The results for Monukka and Alicante Bouschet indicate a similar condition with respect to sugar content of the mature flowers. A decrease in number of leaves to a vine was in each case accompanied by a decrease in the percentage of sugars present.

That the larger percentage of sugars present in the flowers improves their development is indicated by the figures on germinability of pollen. The pollen of the cane-pruned Muscat of Alexandria vines gave a germination of 37 and 38 per cent, that of the normally pruned vines 12 and 11 per cent, and that of the defoliated vines only 3 and 4 per cent, respectively, for 1927 and 1928. The variation in the germination of the pollen of the other varieties was similar to that of the Muscat.

A further indication of the improvement in flower development resulting from the better nutrition is shown in figure 6. This figure shows average clusters of Alicante Bouschet taken from cane-pruned, normally pruned, and defoliated vines just after the berries had set. The clusters from both the cane-pruned and normally pruned vines set a normal crop of berries. The cluster from the cane-pruned vine, however, is much longer owing to the better nutrition it had during the later stages of its development. The average length of cluster of the cane-pruned vines was $22.3 \pm .05$ centimeters, while that of the

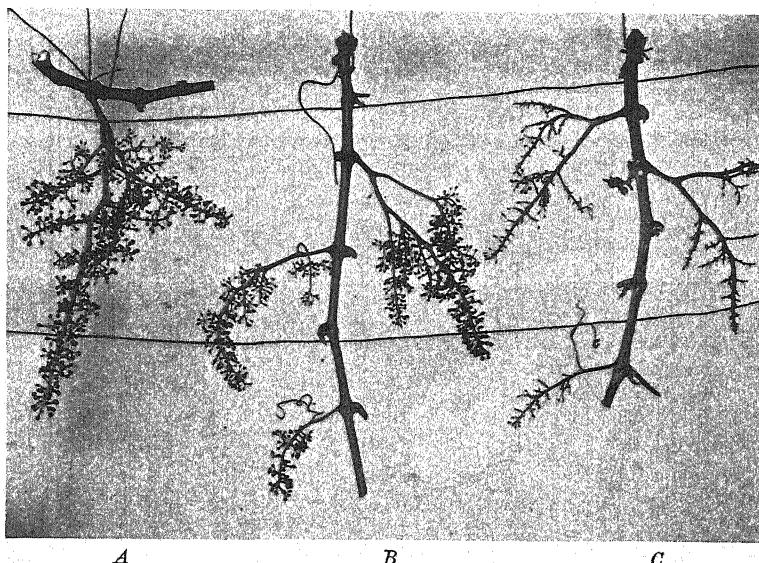


Fig. 6. The effect of the number of leaves to a vine on the size of the clusters and the setting of the berries in Alicante Bouschet. A, Cane pruned, flower-cluster thinned. B, Normally pruned. C, Normally pruned, defoliated.

normally pruned vines was only $15.5 \pm .03$ centimeters. On the contrary, the clusters from the defoliated vines are shorter than those of the vines with leaves and only a few berries set. Both of these conditions may be attributed to poorer nutrition during the later stages of development of the clusters. In addition to the influence of the low percentage of sugars present in the flowers under this treatment on the development of the male parts of the flower, as shown by the germinability of pollen, there is indication that the female parts of the flower, too, are weakened. Although the pollen of these flowers was poor, there were vines with good pollen only twelve feet away which should have given these flowers, had they been susceptible to fertilization, a chance to set berries.

In the case of Monukka, not a single berry was set on the defoliated vines in either 1927 or 1928, even though their pollen gave a germination of 5 per cent. This further indicates that the female as well as the male parts of the flower are weakened by the poorer nutrition of these vines.

The Effect of Nutrition on Berry Setting and Development.—During a number of seasons data has been collected on the effect of nutrition, as influenced by the number of leaves to a vine, on the setting of the berries and further development of the clusters. The data for the seasons of 1927 and 1928 are given in table 4.

TABLE 4
THE EFFECT OF THE NUMBER OF LEAVES TO A VINE ON THE SETTING OF THE BERRIES AND THE DEVELOPMENT OF MUSCAT OF ALEXANDRIA CLUSTERS

Treatment	Leaves to a vine	Crop to a vine (kilos)	Clusters			Number to a cluster	Normal berries	Average weight of all berries, grams
			Number to a vine	Average weight, grams	Average length, cm.			
Non-pruned, with flower-cluster thinning.....	3,400+	25.9±0.66	58	474±17	28.0±0.5	116±4.2	93	4.1±0.06
Cane pruned, with flower-cluster thinning.....	1,700	15.1±0.36	38	430±24	27.0±0.4	124±5.2	92	4.2±0.07
Normally pruned (crop limited by pruning).....	760	13.7±0.57	62	211±13	20.8±0.5	45±2.6	68	3.8±0.09
Severely pruned (crop reduced by pruning).....	540	8.9±0.41	44	200± 9	20.0±0.6	35±1.7	63	3.1±0.11
Normally pruned, defoliated ^a 2 weeks before blooming....	0	5.8±0.25	53	105±11	19.0±0.4	20±2.2	52	1.8±0.07

* Leaves arising after the berries had set were allowed to develop.

The figures of table 4 show a positive correlation between number of leaves to a vine and the setting of the berries and the development of the fruit. In each of the measurements made there was a deterioration in the clusters with a decrease in the number of leaves to a vine, except in two measurements in going from the non-pruned to the cane-pruned vines. In these cases the very much larger crop of the non-pruned vines somewhat counteracted the beneficial influence of the increase in the number of leaves to a vine.

The decrease of crop below that of the non-pruned vines was 41 per cent for the cane-pruned vines, 47 per cent for the normally pruned vines, 65 per cent for the severely pruned vines, and 77 per cent for the defoliated vines. The differences in the crop of the cane-pruned and non-pruned vines and that of the vines with other treatments was primarily the result of the poorer setting of the berries on the vines with fewer or no leaves.

The effect of the several treatments on the setting of the berries is shown by the number of normal berries to a cluster. The set of normal berries to a cluster on the normally pruned vines was 175 per cent smaller than on the cane-pruned vines, and 158 per cent smaller than on the non-pruned vines. Severe pruning and defoliation two weeks before blooming reduced the number of normal berries to a cluster to 22 and 35 per cent, respectively, below that of the normally pruned vines.

Accompanying the decrease in the number of normal berries to a cluster, as the number of leaves to a vine became less, there was a decrease in both the weight of cluster and the percentage of normal berries to a cluster. The clusters of the normally pruned vines weighed 104 per cent less than those of the cane-pruned vines, and 125 per cent less than those of the non-pruned vines. Severe pruning and defoliation reduced the weight of clusters below that of the clusters of the normally pruned vines by 5 and 50 per cent respectively. The percentage of normal berries to a cluster was reduced by a decrease in the number of leaves to a vine in a similar manner to weight of cluster, the magnitude of the reduction being somewhat less.

The clusters of the cane-pruned and non-pruned vines were 35 and 40 per cent, respectively, longer than those of the normally pruned vines. Severe pruning and defoliation have had little or no effect on length of cluster. This might be expected with defoliation, since the clusters attain their full length before the flowers are mature or before blooming, and hence before the leaves were removed from the vines which were defoliated.

Despite the smaller crop and small number of berries to a cluster, there was a decrease in the average weight of berry with each decrease in the number of leaves to a vine. The berries of the cane-pruned and non-pruned vines were 10.5 and 8 per cent, respectively, heavier than those of the normally pruned vines, and severe pruning and defoliation reduced the weight of berry under that of normal pruning by 18.4 and 52 per cent respectively.

The beneficial influence of a large number of leaves to a vine on the size of cluster and the development of the berries on Muscat of Alexandria is further shown in figures 7, 8, and 9. As indicated in figures 7A and 8, the clusters of the cane-pruned vines are well filled with normal berries of uniform size. Figures 7B and 9 show that the clusters of the normally pruned vines are affected by coulure and that the berries are irregular in size, there being a considerable number of shot berries on each cluster. The cluster from the defoliated vines

fig. 7C coulured badly and show an increased tendency to *millerandage*—the setting of small seedless (shot) berries. On these latter clusters the normally seeded berries are also small.

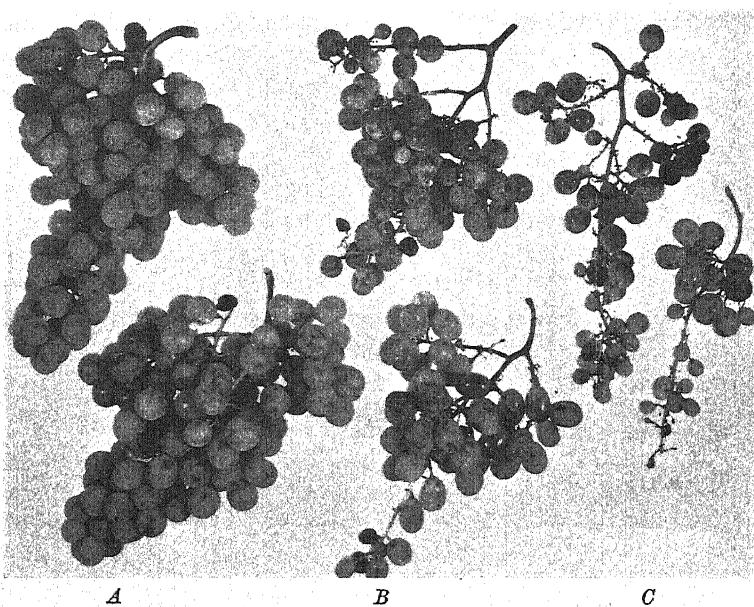


Fig. 7. The effect of the number of leaves to a vine on the size of cluster and the setting and development of the berries of Muscat of Alexandria. A, Cane-pruned, flower-cluster thinned. B, Normally pruned. C, Normally pruned, defoliated.

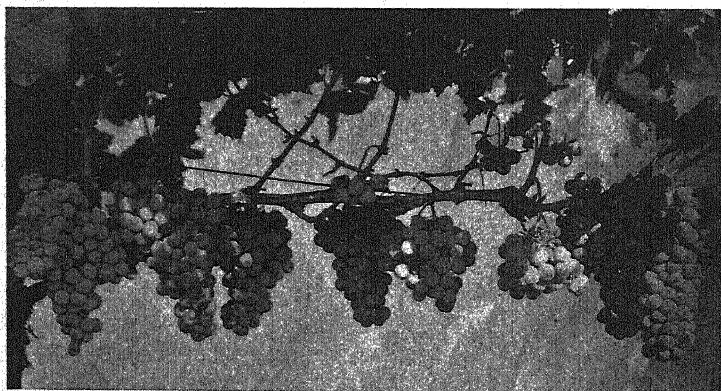


Fig. 8. An excellent crop of fruit on a single cane of a Muscat of Alexandria vine that was cane-pruned and on which the crop was controlled by flower-cluster thinning.

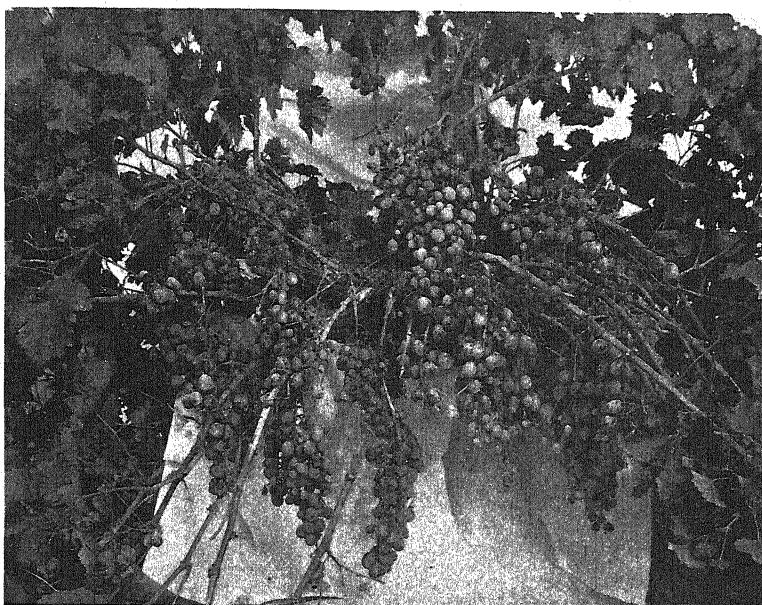


Fig. 9. The crop of a representative Muscat of Alexandria vine that was normally pruned.

SUMMARY

With Decreased Pruning the Amount of Carbohydrates Increases.—In the normal seasonal changes of the total carbohydrates of the vine there are two maxima and two minima. One of the maxima occurs in the dormant season and the other occurs during early summer.

With a decrease in pruning the amounts of total carbohydrates present were increased at each maximum, the percentage increase being greater at the maximum in early summer. The severely pruned vines failed to show a maximum in the seasonal trend of carbohydrates during early summer.

The larger amount of carbohydrates present during early summer with decreased pruning is primarily if not solely confined to the carbohydrates in solution—sugars. The amount of reducing sugars in the non-pruned vines of Muscat of Alexandria was 52 per cent larger than in the normally pruned vines on June 3, and the amount of total sugars 51 per cent larger.

The effect of pruning on the amounts of carbohydrates present during early summer was most pronounced in the shoots. The

amounts of these materials present in the trunk and in the roots were influenced very little.

With Decreased Pruning the Growth is more Favorable to the Production and Accumulation of Carbohydrates.—The non-pruned vines contain three or four times as much reducing sugars and total carbohydrates at the beginning of the growing season than the normally pruned vines.

During early summer, the ratio of weight of shoot growth to weight of vine at the beginning of the growing season is much greater for the severely and normally pruned vines than for the non-pruned vines, while the ratio of weight of leaves to shoots is much less for the severely and normally pruned than for the non-pruned vines.

Total shoot elongation proceeds much more rapidly in the early growing season on the non-pruned than on the severely or normally pruned vines. During late May and June when the rate of total shoot elongation of the severely and normally pruned vines is increasing rapidly, that of the non-pruned vines is moderating. After July 1 the non-pruned vines make very little additional shoot growth, while the shoots of the severely and normally pruned vines continue rapid growth for most of the season.

The difference in rate of total shoot elongation during early summer supplies the non-pruned vines with a weight of leaves several times greater than that of either the severely or normally pruned vines. This is equivalent to a considerable increase in the season of leaf activity for the non-pruned vines.

With Decreased Pruning the Rates of Growth and of Carbohydrate Nutrition are more Favorable to Fruiting.—Increases in number of leaves, especially during early summer, increased the percentages of reducing sugars in the mature flowers. In Muscat of Alexandria the amounts of reducing sugars in the flowers of the normally pruned vines with leaves was from 10 to 80 per cent larger than in the defoliated vines and the amount of total sugars 22 to 56 per cent larger. The amounts were still larger in the cane-pruned vines. The results with Monukka and Alicante Bouschet indicate a similar relation of leaves to increased sugar content of the mature flowers.

Accompanying the increase in the sugar content of the mature flowers there was a very marked increase in germinability of pollen. The germinability of the Muscat of Alexandria pollen was 3 and 4 per cent on the defoliated, 12 and 11 per cent on the normally pruned vines with leaves, and 37 and 38 per cent on the cane-pruned vines, respectively, during 1927 and 1928.

The figures on germinability of pollen indicate a positive correlation between carbohydrate nutrition and the development of the male parts of the flower. Figures on the setting of the berries show a similar correlation between carbohydrate nutrition and the development of the female parts of the flowers.

A decrease in the number of leaves to a vine (see table 4) has been accompanied by a smaller capacity for production, by a decrease in weight of cluster, a decrease in the number of normal berries to a cluster, a decrease in percentage of normal berries to a cluster, a decrease in the length of cluster, and a decrease in the weight of berry. There was, moreover, a great increase in the tendency to coulure in each of the varieties used, and to *millerandage*, especially in the Muscat of Alexandria.

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FACTORS WHICH MODIFY THE RESISTANCE OF WHEAT TO BUNT, *Tilletia tritici*^{1, 2}

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In an earlier publication^{(2) 4} the author presented data which indicated that Martin wheat differs from such susceptible varieties of wheat as White Federation and Hard Federation in one main dominant factor for resistance to bunt, *Tilletia tritici*, and that Hussar wheat differs from these susceptible varieties in two main factors for resistance, one of which was shown to be identical with the Martin factor.

Because some susceptible plants nearly always escape infection it was necessary to classify F_2 on the basis of percentages of bunt in F_3 rows which were grown from the seed of individual F_2 plants. Although Martin and Hussar were completely free from bunted plants, there were not enough bunt-free F_3 rows to make up the resistant classes. A few rows with a low percentage of bunt had to be included in the resistant groups. At that time it was pointed out that the presence of a few diseased plants in resistant rows might be due to modifying factors. Also it was suggested that genetically resistant plants occasionally might become infected. Data now are available

¹ Cooperative Investigations of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Agronomy, California Agricultural Experiment Station.

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⁴ Superscript numbers in parentheses refer to "Literature Cited," page 184.

which show that the first and probably the second of the above conditions prevail, in addition to the fact that the one or two diseased plants in resistant rows occasionally may result from mechanical mixtures.

METHODS AND MATERIALS

Plants were selected from rows showing between 1 and 5 per cent of bunt, with the view of obtaining selections which would breed true for a similar low percentage of bunt. Bunted plants occurring in such rows rarely are completely bunted. Usually such plants will have two or more sound heads, with the result that seeds are available for propagation.

The selections were grown in the field at University Farm, Davis, California. Conditions there favor such investigations, because relatively high bunt infection can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winter-killing, and with the assurance that both types will mature in the following summer.

The seeds were thoroughly blackened with bunt by placing an excess quantity of the spores with the wheat in a glass container and shaking vigorously. The inoculum, *Tilletia tritici*, was collected by Professor W. W. Mackie in 1917, on Little Club wheat in the Montezuma Hills district of Solano County, California. This collection was originally propagated by Professor Mackie on Little Club wheat, in the Botany Garden at Berkeley, California. Since 1919 the writer has propagated bunt from this same collection on White Federation wheat at Davis. The inoculum used, therefore, has been derived from one original collection of bunt. This procedure was followed at first, not because it was suspected that there were physiologic forms of bunt, but because this method offered an accessible and definite source of spores. Physiologic forms of this fungus now are known to exist.^(5, 6, 7, 8) The fact that the same collection of bunt has been used continuously at Davis makes it reasonably certain that the same form or possibly mixture of forms has been employed in all the writer's investigations. This fact is indicated also by the constant way in which the parental wheat varieties have reacted to this inoculum.

The seeds were spaced from 2 to 3 inches apart in rod rows one foot part. The entire nursery was sown within 3 or 4 days in order to avoid the effects of different temperatures and soil moistures. The nursery always was sown in a field where no wheat had been grown during the previous year, so that it was almost entirely free from volunteer grain.

At harvest time the plants in each row were pulled and separated into two piles: bunt-free and bunted. The total number of plants and the number of bunted plants were recorded and the percentage of infected plants calculated. A plant was classified as bunted if it showed any infection.

The selections all were made from a cross of Hussar with Hard Federation. Hussar is one of the two varieties of wheat found to remain entirely free from bunt when inoculated with spores of *Tilletia tritici* in an extensive test conducted by the Washington, Oregon, and California Agricultural Experiment Stations in cooperation with the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.⁽⁶⁾ Hussar has been free from bunt over a 9-year period when inoculated with the bunt collection used in these experiments. It has been attacked by some collections of bunt used elsewhere.^(6, 7) Hard Federation is very susceptible to bunt under the conditions of these experiments, as may be seen from the data in table 1.

TABLE 1

ANNUAL PERCENTAGES OF BUNTED PLANTS IN HUSSAR AND HARD FEDERATION WHEATS IN THE 9 YEARS FROM 1920 TO 1928, AT DAVIS, CALIFORNIA

Variety	Percentage of bunted plants									
	1920	1921	1922	1923	1924	1925	1926	1927	1928	9-year average
Hussar.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hard Federation.....	89.7	62.4	53.3	61.4	82.1	59.8	83.7	84.2	84.6	73.4

There is considerable variation in the percentage of bunted plants in Hard Federation from year to year. Similar fluctuations are exhibited by other varieties, and probably are due to seasonal differences. During the nine years, a total of about 100 rod rows of Hussar and Hard Federation have been grown.

The selections used in these experiments were made from F_4 rows of a cross of Hard Federation with Hussar, made in 1921, for the purpose of studying the inheritance of resistance to bunt. Only the four selections which have been studied most extensively will be considered in this paper. Four rather certain cases of mechanical mixtures occurred in these experiments. It was easy to detect mechanical mixtures because the most extensive plantings were made in F_6 and F_7 , and the families therefore were homogeneous for most visible characters. There was a little evidence of field hybridization, but the data are fragmentary and therefore will not be presented.

EXPERIMENTAL RESULTS

The data may be examined to determine whether the occurrence of a few bunted plants in a resistant row is due (a) to the fact that genetically resistant plants occasionally may become infected, or (b) to the presence of modifying factors.

TABLE 2

PERCENTAGE OF BUNTED PLANTS PRODUCED BY SELECTIONS FROM ROW 478 OF THE F₄ OF HARD FEDERATION X HUSSAR WHEATS

1925 F ₄		1926 F ₅		1927 F ₆		1928 F ₇	
Row	Bunted, per cent	Row	Bunted, per cent	Row	Bunted, per cent	Row	Bunted, per cent
32	0.0	2	0.0	3	0.0	18	0.0
		3	0.0	4	0.0	19	0.0
		4	0.0	5	0.0	20	0.0
		5	0.0	6	0.0	21	0.0
		6	0.0	7	0.0	22	0.0
		7	0.0			23*	0.0
		8	0.0			24	0.0
		9	0.0			25	0.0
		11	0.0			26	0.0
		12	0.0			27	0.0
		13	0.0			28	0.0
		14	0.0			29	0.0
		15	0.0			30	0.0
		16	0.0			31	0.0
		17	0.0			32	0.0
		18	0.0			33	0.0
		19	0.0			34	0.0
		20	0.0			35	0.0
		21	0.0			36	0.0
		22	0.0			37	0.0
		23	0.0			38	0.0
		24	0.0			39	0.0
		25	0.0			40	0.0
		26	0.0			41	0.0
		27	0.0			42	0.0
		28	0.0			43	0.0
		29	0.0			44	0.0
		30	0.0			45	0.0
478 Hard Federation x Hussar	2.8					46	0.0
		33*	0.0			47	2.7
						48	0.0
						49	0.0
						50	0.0
						51	0.0
						52	0.0
						53	0.0
						54	0.0
						55	0.0
						56	0.0
						57	0.0
						58	0.0
						59	0.0
						60	0.0

* Row sown from seed of partly bunted plants.

In 1925, row 478 contained one partly bunted plant in a total of 38, or 2.8 per cent of bunted plants. The results obtained by propagating the partly bunted plant and one of the bunt-free plants may be seen in table 2.

In 1927 four rows produced a considerable percentage of bunted plants, but they were so obviously different in morphologic characters that they were discarded as rogues. The good seed produced by the partly bunted plant in 1925 were sown in row 33 in 1926, but no bunted plants resulted. The seeds from 27 plants were sown in 1927. One row contained one partly bunted plant, the seeds from which produced all healthy plants in 1928. The seed from nine sibs likewise gave rise to no diseased plants.

A bunt-free plant selected from row 478 in 1925 and propagated in row 32 in 1926 produced no bunted plants. Forty-three selections of its descendants grown in 1927 and 1928 also were free from bunt.

Certainly, the two rows which produced bunt in the above experiment are nearly, if not quite, as resistant as their sibs. For practical purposes these rows might be considered as resistant genetically as Hussar, even though the one row containing bunt in 1927 was a direct descendant of the partly smutted plant produced in 1925. If the smut appearing in this line is due to modifying factors, the effect is very slight.

Numerous selections have been grown for two or more years without the production of a single bunted head. In fact, the majority of resistant selections obtained from crosses of Martin and Hussar with susceptible varieties resemble the resistant parents in that they never produce any bunt.

If the low percentage of bunt in resistant F_3 rows is due to the action of modifying factors, it should be possible to select lines of wheat which would breed true for this character. A selection was sought which would allow less than 5 per cent of bunt to develop. That such a selection has been obtained may be seen from the data in table 3.

Row 1415 contained one partly bunted plant in 1925. Seeds from this partly smutted plant and from one bunt-free plant were sown and plants grown in the following year. Row 112, from the seed of the partly bunted plant, contained 2.2 per cent of bunted plants in 1926. Row 111, from the seeds of the bunt-free plant, contained no bunted plants. However, in 1927, the progenies from plants of row 112 contained an average of 3.2 per cent of bunted plants. Similar progenies from plants of the bunt-free row 111 contained an average

TABLE 3

PERCENTAGE OF BUNTED PLANTS PRODUCED BY SELECTIONS FROM F₄ ROW 1415 OF
HARD FEDERATION X HUSSAR

1925 F ₄		1926 F ₅		1927 F ₆		1928 F ₇	
Row	Bunted, per cent	Row	Bunted, per cent	Row	Bunted, per cent	Row	Bunted, per cent
111	0.0	62	1.7	43	0.0	62	0.0
		63	3.8	44	0.0	63	0.0
		64	5.7	45	3.1	64	0.0
		65	2.2	46	0.0	65	0.0
		66	2.9	47	0.0	66	0.0
		67	5.7	48	0.0	67	0.0
		68	3.8	49	0.0	68	0.0
		69	2.0	50	0.0	69	0.0
		70	7.5	51	1.5	70	1.5
		71	0.0	52	0.0	71	0.0
		72	0.0	53*	1.8	72	0.0
		73	5.9	54*	2.4	73	1.8
		74	0.0	55*	1.7	74	2.4
		75	2.1	56	1.7	75	1.7
		76	0.0	57	0.0	76	0.0
		77	5.9	58	0.0	77	0.0
		78	0.0	59	0.0	78	0.0
		79	0.0	60	0.0	79	0.0
		80	4.3	61	2.3	80	0.0
		81	15.0	62	0.0	81	2.3
		82	0.0	63*	0.0	82	0.0
		83	0.0	64*	AV.--0.5	83	0.0
		84	0.0	65	0.0	84	0.0
		85	6.7	66	0.0	85	0.0
		86	0.0	67	0.0	86	0.0
		87	5.1	68	1.7	87	1.7
		88	1.9	69	0.0	88	0.0
		89	0.0	70	0.0	89	0.0
		90	2.3	71	0.0	90	0.0
				72	0.0	91	0.0
				73	0.0	92	0.0
1415 Hard Federation x Hussar	3.7			74*	AV.--0.2	93	0.0
		112*	2.2	75	0.0	94	1.5
				76	0.0	95	0.0
				77	0.0	96	0.0
				78	0.0	97	0.0
				79	0.0	98	0.0
				80	0.0	99	0.0
				81	3.2	100	0.0
				82	1.7	101	1.6
				83	4.0	102	0.0
				84*	AV.--0.5	103	0.0
				85	0.0	104	11.5
				86	0.0	105	0.0
				87	0.0	106	2.9
				88	0.0	107	0.0
				89	0.0	108	2.6
				90	0.0	109	0.0
				91	0.0	110	0.0
				92	0.0	111	3.3
				93	0.0	112	1.9
				94*	AV.--1.1	113	2.2
				95*	0.0	114	0.0
				96*	0.0	115	0.0
				97*	0.0	116	7.7
				98*	0.0	117	4.2
				99*	2.1	118	2.8
				100	1.9	119	10.1
				101	0.0	120	4.71
				102	0.0		AV.--3.2
				103	0.0		
				104	0.0		
				105	3.4		
				106	0.0		
				107	2.2		
				108	0.0		
					AV.--0.9		

* Rows planted from the good seeds of partly bunted plants.

of 2.9 per cent of diseased plants. Similar results were obtained in 1928, but the actual percentages of bunted plants were lower.

In 1927, selections were made from bunt-free rows, from rows containing about 2 per cent of bunt, and rows containing near the maximum percentage of bunt produced in that year. In general, selections from rows containing no diseased plants appear to produce a lower average percentage of bunt than selections from rows containing the higher percentages of bunt. Also populations from partly bunted plants usually have a higher average percentage of bunt than populations from bunt-free sibs. However, all the selections from row 111, of 1926, contained an average of 1.15 per cent of bunt in 1927 and 1928, as compared with 1.20 per cent in selections from row 112, which was grown from the seed of a partly bunted plant.

The variation in the percentage of bunt in rows from the same parent was greater in 1927 than in 1928. This suggests that these differences probably are due to environmental influences. The fact that rows containing the higher percentages of bunt in 1927 usually produced progeny having slightly higher percentages in 1928 might indicate that a greater number of modifying factors are present in these rows, or possibly that factors with a greater effect are involved. Because of environmental influences it is impossible to get an exact expression of the influences of these factors, and therefore it is undesirable to draw too fine distinctions.

That the effects of modifying factors may be considerably greater than those just considered may be seen from the data in table 4.

In 1925 row 1417 contained one partly bunted plant. Plants from the seeds of this partly bunted plant and from one bunt-free plant were grown in 1926, and their progenies in 1927. The partly bunted selection (row 117) gave rise to lines very similar to those shown in table 3. However, the bunt-free selection (row 116) produced one partly bunted plant out of a total of 21, or 4.8 per cent. Plants from the seeds of this partly bunted plant, together with those from 16 bunt-free plants, were grown in 1927. The row (No. 152) from the seeds of the partly bunted plant contained 40 per cent of bunted plants. The rows from the bunt-free plants produced from 0 to 25 per cent of bunt in 1927. Selections from row 165, with no bunt, and row 158, with 1.9 per cent of bunt, produced lines in 1926 similar to those already considered in table 3.

Selections from row 156, which contained 22.9 per cent of bunted plants, produced an average of 5.1 per cent of diseased plants in 1928. The 22.9 per cent of bunted plants in row 156 at first suggested that it must have resulted from a natural cross with some susceptible plant

TABLE 4

PERCENTAGE OF BUNTED PLANTS PRODUCED BY SELECTIONS FROM F₄ ROW 1417
OF HARD FEDERATION X HUSSAR

Row	1925 F ₄		1926 F ₅		1927 F ₆		1928 F ₇	
	Row	Bunted, per cent						
116	4.6							
152*	40.0						129*	12.0
153	9.9						130*	0.0
154	25.0						131*	1.4
155	9.5						132*	3.3
156	22.9						133*	3.3
157	11.1						134*	10.0
158	1.9						135*	5.2
159	20.0						136*	10.8
160	16.9						137*	18.9
161	1.6						138*	7.3
162	5.0						139*	1.5
163	8.7						140*	7.4
164	18.5						141	1.7
165	0.0						142	1.5
166	12.5						143	0.0
167	14.2						144	0.0
168	6.3						145	1.6
Av.--13.3							146	2.6
1417 Hard Federation x Hussar		4.0					147	0.0
117*	2.1						148	13.0
169*	3.3						149	Av.--5.1
170	2.6						150	0.0
171	6.2						151	0.0
172	0.0						152	0.0
173	3.3						153	0.0
174	3.0						154	0.0
175	7.1						155	0.0
176	0.0						156	0.0
177	0.0						157	0.0
178	7.7						158	3.5
179	4.2						159	0.0
180	1.6						160	0.0
181	0.0						161	3.4
182	0.0						162	1.5
183	6.7						163	1.8
184	7.9						164	0.0
185	0.0						165	0.0
186	2.7						166	0.0
187	1.6						167	0.0
188	0.0						168	0.0
189	13.0						169	0.0
190	3.6						170*	0.0
191	14.8						171	0.0
192	0.0						172	0.0
193	1.8						173	0.0
194	0.0						174	0.0
195	1.6						175	0.0
196	0.0						176	0.0
197	2.1						177	0.0
198	0.0						178	0.0
Av.--3.2							179	0.0
190*							180*	Av.--0.5
181*							182*	0.0
182*							183*	1.6
183*							184*	1.3
184*							185	0.0
185							186	0.0
186							187	3.3
187							188	0.0
188							189	0.0
189							190	0.0
190							191	8.1
191							192	1.6
192							193	0.0
193							194	Av.--1.7

*Row planted from the good seeds of partly bunted plants.

and was heterozygous for resistance. However, it differed in one important respect from heterozygous rows previously observed. In heterozygous rows resulting from a cross between a susceptible and a resistant wheat from a third to a half or more of the diseased plants are completely bunted, and consequently produce no seed. On the other hand, all the diseased plants in row 156 were only partly bunted, and seeds from them were used for growing plants in 1928. In fact, almost without exception the diseased plants in all the selections in these experiments were partly bunted. That row 156 did not come from a heterozygous plant will be seen from the results obtained in 1928. Because considerably more bunt was produced in this family than in any of the other families, the results suggest that probably a larger number of modifying factors are present, or perhaps that the modifying factors present exert a greater effect.

DISCUSSION AND CONCLUSION

It is beyond the scope of this paper to review or discuss in any detail the literature dealing with modifying factors. The effects of modifying factors are frequently met with in genetical experiments, and they have been referred to on numerous occasions in the literature.

Serious attention first was attracted to modifying factors by the controversy in genetic circles arising over the belief that the hooded factor in rats was very unstable. Starting with a common foundation stock, Castle,⁽³⁾ by selecting in the minus direction, practically eliminated the colored areas, whereas in the plus direction he produced a race which was almost self-colored. As Castle had previously found that hooded pattern differed from self-color in a single recessive factor, he believed that the selection experiments showed that selection had altered the hooded factor. The adherents to the multiple-factor hypothesis, on the other hand, held that the hooded factor simply is necessary for the development of the hooded pattern, but that the degree of pigmentation may be modified by numerous other factors. Castle⁽⁴⁾ himself eventually demonstrated the correctness of the multiple-factor hypothesis.

In general, the effects of modifying factors are seen most frequently in connection with quantitative characters. In such material it is difficult to distinguish clearly between the actions of these factors and the effects of environment. Consequently, the study of modifying factors has been an unattractive field. In practically all cases nothing definite is known about them beyond the fact that they do exist.

Bridges⁽¹⁾ seems to have reported the only extensive study of modifying factors. In *Drosophila* studies he found eight specific modifiers of eosin eye color. He not only demonstrated the existence of modifying factors for eosin eye color, but also studied them individually, showing that they differ in no essential respects from other factors. Also he located some of them on the chromosome map.

The data presented in this paper demonstrate the presence of factors which modify the resistance of wheat to bunt. This information should make possible a more complete understanding of the results obtained when a bunt-resistant wheat is crossed with a susceptible one. The occurrence of a low percentage of bunt in resistant F₃ rows is explained adequately. Furthermore, these data suggest a possible explanation for a part of the variability met with in rows of other genotypes. Heterozygous and homozygous susceptible F₃ rows vary considerably in the percentage of bunt which they contain. No doubt a considerable amount of this variation is due to environmental influences. However, it is reasonable to suppose that a part of it may be due to modifying factors.

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AN EXPERIMENTAL STUDY OF TESTS FOR THE DETECTION OF CARRIERS OF BACTERIUM PULLORUM

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INTRODUCTION

A review of the literature of the last decade shows that investigators are far from being in unanimous agreement regarding the value of tests for the detection of fowls that are carriers of *Bacterium pullorum*. Following the findings of Jones⁽¹⁾ in 1912, that the agglutination test was of value for this purpose, this test came into extensive use in some sections of the country.

The rather complicated procedure of performing the agglutination test has undoubtedly been an important factor in retarding its more general employment throughout the United States for the eradication of *Bacterium pullorum* infection in breeding fowls. In addition, the accuracy of the test has recently been questioned by several investigators, particularly B. A. Beach, Halpin, and Lampman,⁽²⁾ J. R. Beach,⁽³⁾ Fitch and Lubbehusen,⁽⁴⁾ and Newsom, Cross, and Ufford,⁽⁵⁾ who have shown conclusively that a negative reaction to the agglutination test does not always indicate freedom from *Bacterium pullorum* infection. These factors have stimulated attempts to develop a test of equal or greater accuracy but simpler in application than the agglutination test.

In 1917, Ward and Gallagher⁽⁶⁾ reported upon an intradermal test for detecting the carriers of *Bacterium pullorum*. These investigators used a broth culture of *Bacterium pullorum*, which was incu-

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bated for about a month, carbolized, and stored for several weeks longer. This was injected intradermally into the margin of the wattle. A positive reaction consisted of a swelling of the injected wattle which persisted for at least twenty-four hours. Similar experiments have since been conducted by a number of investigators, including Scherago and Benson,⁽⁷⁾ J. R. Beach,⁽⁸⁾ Fuller,⁽⁹⁾ Brunett,⁽¹⁰⁾ Cernaianu,⁽¹¹⁾ Edington,⁽¹²⁾ Broerman,⁽¹³⁾ Graham and Tunnicliff,^(14, 15) Edwards and Hull,⁽¹⁶⁾ Gwatkin,⁽¹⁷⁾ Stafseth and Thorp,⁽¹⁸⁾ Bushnell and his associates,^(19, 20, 21) B. A. Beach,⁽²²⁾ and Owen.⁽²³⁾ Interest in the intradermal test was further stimulated in 1926 and 1927, when various manufacturers of biologic preparations began marketing, under the name of 'pullorin' products for testing poultry for *Bacterium pullorum* infection by the intradermal method. Variable results have been reported by the different investigators, but their findings for the most part indicated that the intradermal test was less satisfactory than the agglutination test.

With few exceptions, the experiments previously mentioned were concerned with one type of preparation for intradermal use and, consequently, studies of the relative value of different types of such preparations have been lacking. Furthermore, the result of the agglutination test has been the principal criterion of the merits of the intradermal test, whereas this should more properly consist of the bacteriological findings alone. It was with these considerations in mind that the studies reported herein were undertaken.

METHODS USED IN THE EXPERIMENTS AT THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

In these experiments four types of preparation for intradermal testing were used, i.e., concentrated, precipitated, cell suspension, and cell solution. For the sake of brevity, these will be designated by the proprietary name of 'pullorin'. Part of the pullorins employed were secured from outside sources and part were prepared in our laboratory. Some of the fowls that were tested came from commercial flocks; others were of those used at the Experiment Station. All the birds were subjected to the intradermal and agglutination tests simultaneously and were later destroyed for post-mortem and bacteriological examinations.

The pullorin was injected intradermally into the lower margin of the right wattle. An attempt was made to produce a swelling, about the size of a wheat kernel, at the point of injection. The readings

were made after twenty-four hours. The scheme of reading intradermal reactions followed by Graham³ in his experiments with the pullorin test was used. This is illustrated in figure 1.

The agglutination tests were set in dilutions of 1-25, 1-50, 1-100, and 1-200. Complete agglutination in any one of the four tubes was considered as a positive reaction.⁴

Upon completion of the intradermal and agglutination tests, the fowls were killed and examined. Lesions occurring in any organ were noted and cultured and, in addition, cultures were made of heart

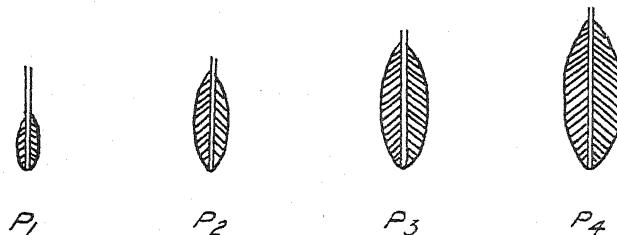


Fig. 1. Illinois Experiment Station diagram of reactions to pullorin. The clear center space indicates the thickness of a normal wattle. The shaded portion indicates the swelling resulting from the injection of pullorin.

blood, liver, and ovaries (of hens) or testicles (of males), whether or not lesions were present. Each culture was made in duplicate in bouillon and cooked blood agar. All the cultures which presented Gram-negative rods were planted in glucose, mannite, maltose, saccharose, and lactose broth, each containing 1 per cent Andrade's indicator. Those that fermented glucose and mannite only were recorded as *Bacterium pullorum*; strains producing acid alone were termed anaerogenic, those producing both acid and gas, aerogenic.

Only pullorin of the precipitated or powdered type was secured from an outside source. This was dissolved in sterile water or sterile water containing 0.5 per cent phenol, just before use. Part of the pullorin was purchased from a commercial laboratory and part was furnished by Dr. Robert Graham of the Illinois Agricultural Experiment Station.

³ Graham, R., Personal communication, 1928.

⁴ Technique of the agglutination test.—A culture (No. 9) of *Bacterium pullorum* of known good agglutinating qualities was used for the antigen for all of the tests. This antigen was prepared by washing off a 48-hour growth of *Bacterium pullorum* in agar flasks with 0.85 per cent saline solution containing 0.5 per cent phenol; 1 cc of N₁ NaOH was added to 100 cc of antigen, according to Mallman's⁽²⁴⁾ recommendation, to eliminate cloudy reactions. It was standardized to the density of 4.5 cm by the Gates' nephelometer.

The tubes were incubated at 37° C for 24 hours. First readings were made after this period. They were then held at room temperature for another twenty-four hours and, at the termination of this time, the final reading was made.

TESTS WITH THE COMMERCIAL PULLORIN

Eighty-five white leghorn hens, secured from a poultryman, were tested with this product and by the agglutination test, and were killed and examined after the tests were completed. All of the hens had previously reacted to an agglutination test. The results are given in table 1.

TABLE 1

COMPARISON OF THE RESULTS OF TESTS WITH A COMMERCIAL PRECIPITATED
PULLORIN AND BY THE AGGLUTINATION METHOD, AND OF POST-MORTEM
EXAMINATION OF 85 HENS

Results of pullorin tests	Results of agglutination tests	Macroscopic lesions present	Bacterium pullorum isolated	Number of fowls	Percent of total fowls
+	+	+	+	52	61.1
+	+	+	-	2	2.3
+	+	-	-	1	1.1
+	-	+	-	3	3.5
+	-	-	-	5	5.8
-	+	+	+	18	21.1
-	-	-	-	4	4.7

¹ The reactions of 35 fowls were recorded as P₁, of 13 as P₂, and of 4 as P₃. The swelling involved both wattles of one of the P₃ fowls.

² The reaction of 1 fowl was recorded as P₁, of 1 as P₂.

³ Abnormal yolks found in one, fibrinous pericarditis in the other.

⁴ The reaction of this fowl was recorded as P₁.

⁵ The reactions of 2 fowls were recorded as P₁, of 1 as P₂.

⁶ *Bacillus coli* obtained in cultures from abnormal yolks of one bird and from a small abdominal cyst of another. The third bird had an abdominal cyst, the culture from which remained sterile.

⁷ The reactions of all five fowls were recorded as P₁.

The data presented in table 1 show a marked discrepancy between the results of the pullorin tests and the bacteriological findings. Such discrepancy, but much less marked, is also seen with respect to the results of the agglutination test. This is brought out more clearly by further summarizing of the data. Thus, of the 70 bacteriologically positive fowls, 52, or 74.2 per cent, were detected by the pullorin test, while all of them gave a positive reaction to the agglutination test. Of the 15 bacteriologically negative fowls, 11, or 73.3 per cent, reacted to the pullorin test, and 3, or 20 per cent, reacted to the agglutination test. These results indicate that the reactions that resulted from the intradermal injection of this pullorin were, to a large extent, non-specific. Another objectionable feature of the pullorin tests is that with 43 of the 63 positive reactions, the degree of reactions was slight (P₁). This made a definite reading difficult in many cases.

TESTS WITH THE PULLORINS OBTAINED FROM THE ILLINOIS AGRICULTURAL EXPERIMENT STATION

Five lots of powdered precipitated pullorin were received from this source. These were designated as Nos. 27, 28, 30-A, 5400-E, and 5463-F. Two hundred and thirty-five fowls were tested with these pullorins and by the agglutination method, and were later killed for post-mortem examinations.

The fowls consisted partly of hens secured from poultry farms and partly of 3 to 4-months-old White Leghorn cockerels. Some of the hens were reactors from flocks which had been previously tested by the agglutination method and some were birds that were culled from flocks because of non-productiveness. The cockerels were expected to be free from *Bacterium pullorum* infection, but were included when all of a group of hens to be tested had previously reacted to the agglutination test in order to provide adequate controls for the specificity of the reactions resulting from the injection of pullorin. The results are given in table 2.

In table 2, it is seen that there was very close agreement between the results of the tests with pullorins 27 and 28, the results of the agglutination tests, and the results of the post-mortem and bacteriological examinations of the 92 fowls. The results from the other three pullorins, however, were less satisfactory.

Pullorin 30-A detected 18, or 75 per cent, of the 24 bacteriologically positive fowls and caused a reaction in 8, or 21.6 per cent, of the bacteriologically negative fowls. The results of the agglutination test and the bacteriological examination of the same fowls were in perfect agreement.

Pullorin 5400-E caused a reaction in 12, or 92.3 per cent, of the 13 bacteriologically positive fowls and also in 20, or 58.8 per cent, of the 34 bacteriologically negative fowls. One of the bacteriologically negative fowls reacted to the agglutination test, but otherwise the results of the agglutination tests and bacteriological examinations of the 61 birds coincided.

Pullorin 5463-F caused a reaction in all of the 11 bacteriologically positive fowls and also in 15, or 62.5 per cent, of the 24 bacteriologically negative fowls. With the exception of the reaction of one bacteriologically negative fowl, there was perfect agreement between the results of the agglutination tests and the bacteriological findings.

From the preceding, it is seen that there was a marked variation in the performance of the five lots of the same type of pullorin from the

same source. The reactions produced by two of them appeared to be highly specific in detecting carriers of *Bacterium pullorum*. The other three, however, exhibited such a high degree of non-specificity of reaction that they could not be regarded as of much value for that purpose.

TABLE 2

RESULTS OF TESTS WITH ILLINOIS AGRICULTURAL EXPERIMENT STATION PULLORIN, AND BY THE AGGLUTINATION METHOD, AND OF POST-MORTEM EXAMINATION OF 235 FOWLS

Pullorin No.	Number of fowls tested	Results of pullorin tests	Results of agglutination tests	Macroscopic lesions present	<i>Bacterium pullorum</i> isolated	Number of fowls	Per cent of fowls
27	43	+ ¹	+	+	+	24 ²	55.8
		-	+	+	- ³	1 ²	2.3
		-	-	-	-	18 ⁴	41.8
28	49	+ ⁵	+	+	+	22 ²	44.9
		+ ⁶	+	+ ⁷	- ⁷	1	2.0
		+ ⁸	-	+	+	1	2.0
		-	+	+	+	2	4.1
		-	-	-	-	23 ⁴	46.9
30-A	61	+ ⁹	+	+	+	18 ²	29.5
		+ ¹⁰	-	-	-	8 ¹¹	13.1
		-	+	+	+	6 ² , ¹²	9.8
		-	-	-	-	29 ¹³	47.5
5400-E	47 ¹⁴	+ ¹⁴	+	+	+	12	25.5
		+ ¹⁵	+	-	-	1	2.1
		+ ¹⁷	-	-	-	19	40.4
		-	+	+	+	1	2.1
		-	-	-	-	14	29.7
5463-F	35 ¹⁵	+ ¹⁸	+	+	+	11	31.4
		+ ¹⁹	+	+	-	1	2.8
		+ ²⁰	-	-	-	14	40.0
		-	-	-	-	9	25.7

¹ 8 reactions recorded, as P₁, 13 as P₂, 3 as P₃.

² Fowls that had reacted to a previous agglutination test.

³ *Bacillus coli* isolated from abnormal yolks.

⁴ Control cockerels.

⁵ 10 reactions recorded as P₁, 10 as P₂, 2 as P₃.

⁶ Reaction recorded as P₁.

⁷ Lesions consisted of cysts attached to the oviduct. *B. coli* isolated from cysts.

⁸ Reaction recorded as P₂.

⁹ 7 reactions recorded as P₁, 7 as P₂, 4 as P₃.

¹⁰ 4 reactions recorded as P₁, 4 as P₂.

¹¹ Includes 4 hens and 4 control cockerels.

¹² Included 1 old male. Testes small and hard. *Bacterium pullorum* isolated from testes.

¹³ 3 hens and 26 control cockerels.

¹⁴ 4 reactions recorded as P₁, 3 as P₂, 4 as P₃, and 1 as P₄.

¹⁵ Reaction recorded as P₁.

¹⁶ All hens that had been culled because of non-productiveness. Not previously tested.

¹⁷ 9 reactions recorded as P₁, 8 as P₂, 2 as P₃.

¹⁸ 3 reactions recorded as P₁, 4 as P₂, 3 as P₃, 1 as P₄.

¹⁹ Reaction recorded as P₂.

²⁰ 10 reactions recorded as P₁, 4 as P₂.

The degree of reactions produced by these pullorins was, in a majority of instances, sufficient so that the readings could be easily made, 56 of the 88 positive reactions of the bacteriologically positive birds and 19 of the 44 reactions of the bacteriologically negative birds being recorded as P₂ or greater. In this respect, the performance of these pullorins was more satisfactory than that of the commercial pullorin.

TESTS WITH PULLORINS PREPARED AT THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

For these tests four types of pullorin were prepared. These are concentrated, precipitated, cell-suspension, and cell-solution, and are designated as pullorin *A*, *B*, *C*, and *D*, respectively. The methods of preparation are as follows:

Pullorin A (Concentrated).—This was prepared by the method of Graham,⁵ which is similar to the method of preparation of tuberculin. *Bacterium pullorum* was grown in bouillon for a period of two months. At the end of this time, it was tested for purity, killed by heating for one hour at 60° C, filtered through sterile cotton, and the filtrate reduced to one-tenth of its original volume by heating over the water bath at 80° C, and autoclaved for 20 minutes at 15 pounds pressure. To the concentrated pullorin, an equal amount of 0.85 per cent sterile salt solution was added just before making the intradermal test.

Pullorin B (Alcohol Precipitate).—Concentrated pullorin for this purpose was kindly furnished by Dr. F. W. Wood of the Cutter Laboratories, Berkeley. It had been prepared by growing *Bacterium pullorum* in glycerin-bouillon for a period of 3 months. Precipitation was carried out by the method suggested by Graham⁶ in making powdered pullorin. One part of concentrated pullorin was added to 20 parts of absolute alcohol, the precipitate washed three times in absolute alcohol and three times in sulphuric ether, and dried in jars over calcium chloride. For intradermal tests, 20 per cent solution of the powdered pullorin by weight in sterile 0.85 per cent salt solution containing 0.5 per cent phenol was used. The solution was slightly cloudy and amber in color.

Pullorin C (Cell-Suspension).—This product was obtained by washing 48-hour agar cultures of *Bacterium pullorum* in sterile 0.85 per cent salt solution. The organism used was an aerogenic strain (No. 9) originally isolated from a baby chick. After washing and

⁵ Graham, R. Personal communication, 1928.

⁶ Graham, R., Personal communication, 1928.

centrifuging three times the sediment was re-suspended in sterile 0.85 per cent salt solution, containing 0.5 per cent phenol. The heavy suspension was diluted and standardized by means of the Gates' nephelometer. Four dilutions were made with respective densities of 1, 2, 3, and 4 cm. The four bacterial suspensions were placed in the Arnold sterilizer at 60° C. At the end of one hour, they were removed, cooled, and tested for sterility by inoculating a few drops of the suspension into tubes of bouillon and blood agar. These were then incubated at 37° C for 24 hours and were found to be sterile.

Pullorin D (Cell-Solution Product).—The technique in the preparation of this pullorin is the modification by Schoenholz and Meyer⁽²⁵⁾ of the procedure suggested by Zinsser in the preparation of tuberculin and abortin. In the preparation of pullorin, certain deviations were made from the technique of the first two workers. The 72-hour growth of *Bacterium pullorum* on blake bottles containing agar was washed off with 0.85 per cent salt solution, centrifuged and washed three times, and re-suspended in sterile, distilled water. At this time it was tested for purity. The density of the suspension was measured by Gates' method and found to be approximately 2 mm. To this heavy suspension enough normal sodium hydroxide solution was added to bring the reaction to pH 9.2, and it was then shaken for two hours and neutralized with normal hydrochloric acid solution, after which it was placed alternately in crushed solid carbon dioxide and in boiling water. In this manner it was frozen and thawed successively about thirty times. The freezing process was hastened by the addition of ether to the carbon dioxide. The smears of the suspension were examined at this time and the organisms appeared to be broken up and amorphous. In this process of autolysis of the bacterial cells, the suspension which was originally milky-white in color took on a yellowish tinge. It was passed through a Seitz filter. The filtrate was a clear, slightly amber-colored fluid. This was used in the intradermal tests, after first being tested for its primary toxicity. Along with it, pullorins A, B, and C were also tested to determine whether any false reaction might occur in apparently normal birds. These tests consisted in making intradermal tests with the pullorins and agglutination tests of nine normal cockerels. The results are given in table 3.

From table 3, it is seen that the toxicity tests failed to show that any of the pullorins used would produce a non-specific reaction and that the cockerels were negative to the agglutination test and to the bacteriological examination. Since none of the type C pullorins

TABLE 3
RESULTS OF TOXICITY TEST OF PULLORINS

Band No.	Type of pullorin used	Pullorin reaction	Agglutination test reactions				Post-mortem examination	Bacteriological findings
			1-25	1-50	1-100	1-200		
2826	A Concentrated	—	—	—	—	—	—	—
2827	B Precipitated	—	—	—	—	—	—	—
2817 2800	C Cell-suspension density 1 cm	— —	— —	— —	— —	— —	— —	— —
2815	C Cell-suspension density 2 cm	—	—	—	—	—	—	—
2813	C Cell-suspension density 3 cm	—	—	—	—	—	—	—
2810	C Cell-suspension density 4 cm	—	—	—	—	—	—	—
2831 2835	D Cell-solution	— —	— —	— —	— —	— —	— —	— —

caused a non-specific reaction, the one having the greatest density (1 cm) was selected for use in subsequent tests.

Preliminary Tests.—For these tests, artificially infected birds were used. Each bird of three groups of 17 cockerels received 1 cc each of live suspension of *Bacterium pullorum*. Group I was injected intraperitoneally, Group II intramuscularly, and Group III intravenously. Two weeks later, all the cockerels were bled for the agglutination test and were given the intradermal test with pullorins A, B, and C. The intradermal reactions were read after 24 hours. Following this, all the birds were killed and examined. The results are given in table 4.

Table 4 illustrates that while agglutinins were formed in the blood of some of the cockerels, the negative bacteriological and autopsy findings showed that there was no infection present. The failure of pullorins to cause an intradermal reaction in spite of the presence of agglutinins is in accordance with the contention of Baldwin and Krause as quoted by Fleischner, Meyer and Shaw⁽²⁶⁾ "that cutaneous hypersensitivity is never present without a focus"; in other words, an anatomical foothold of infection is necessary to elicit a skin reaction.

TABLE 4

RESULTS OF PULLORIN AND AGGLUTINATION TESTS AND OF POST-MORTEM EXAMINATION OF ARTIFICIALLY-INFECTED COCKERELS

Mode of infection	Type of pullorin	Number of fowls tested	Results of pullorin test	Results of agglutination test	Macroscopic lesions present	<i>Bacterium pullorum</i> isolated	Number of fowls
Intramuscular	A Concentrated	17	—	+	—	—	3
			—	—	—	—	14
Intraperitoneal	B Precipitated	17	—	+	—	—	9
			—	—	—	—	8
Intravenous	C Cell-suspension	17	—	+	—	—	10
			—	—	—	—	7

Pullorin Tests of Adult Hens.—Two hundred and fifty hens were used in this experiment. One hundred forty-six were White Leghorns which had been culled from a commercial flock because of non-productiveness and which had not previously been tested; 19—of which 17 were Rhode Island Reds and 2 White Leghorns—were reactors from a flock that had been previously tested by the agglutination method, and 85 were White Leghorns that had been used for experimental agglutination tests.

In making the intradermal tests, no attention was paid to the previous agglutination records. Hens were bled for agglutination tests simultaneously with the pullorin test. The summary of results obtained with different pullorins and records of agglutination tests with bacteriological findings are presented in table 5.

From the data given in table 5, it is seen that the results of the agglutination tests coincided closely with the bacteriological findings. Of the entire 250 birds tested and examined, disagreements were encountered only in the case of 3 bacteriologically positive birds that failed to react and of 2 bacteriologically negative birds that reacted to the agglutination test.

There was considerable variance in the accuracy of the results from the different pullorins. The concentrated pullorin detected only 4 (44.4 per cent) of the 9 bacteriologically positive birds and caused a reaction in 8 (13.6 per cent) of the 58 bacteriologically negative birds.

The precipitated pullorin failed to detect the 2 birds that were bacteriologically positive, and caused 10 (32.2 per cent) of the 31 bacteriologically negative birds to react.

The cell-suspension pullorin caused a reaction in 13 (92.8 per cent) of 14 bacteriologically positive birds and also in 22 (53.6 per cent) of 41 bacteriologically negative birds.

TABLE 5

COMPARATIVE RESULTS OF TESTS WITH CONCENTRATED, PRECIPITATED, CELL-SUSPENSION, AND CELL-SOLUTION PULLORINS AND BY THE AGGLUTINATION METHOD, AND OF THE POST-MORTEM EXAMINATION OF THE FOWLS TESTED

Type of pullorin	Number of fowls tested	Results of pullorin test	Results of agglutination test	Macroscopic lesions present	<i>Bacterium pullorum</i> isolated	Number of fowls	Per cent of fowls
<i>A</i> Concentrated	67	+ ¹	+	+	+	4	5.9
		+ ²	-	-	-	8	11.9
		-	+	+	+	5	7.4
		-	-	-	-	50	74.6
<i>B</i> Precipitated	33	+ ³	-	-	-	10	30.3
		-	-	+ ⁴	-	2	6.0
		-	-	+	+	1	3.0
		-	-	+	+	1	3.0
<i>C</i> Cell-suspension	65	+ ⁵	+	+	+	12	18.4
		+ ⁶	-	+	+	1	1.5
		+ ⁷	-	-	-	22	33.8
		-	+	+	+	1	1.5
<i>D</i> Cell-solution	85	-	-	-	-	29	44.6
		+ ⁸	+	+	+	14	16.4
		+ ⁹	-	+	+	1	1.1
		+ ¹⁰	-	-	-	1	1.1
		-	+	-	-	2	2.3
		-	-	-	-	67	78.8

¹ 3 reactions recorded as P₁, 1 as P₂.² 6 reactions recorded as P₁, 2 as P₂.³ 9 reactions recorded as P₁, 1 as P₂.⁴ Abnormal yolks in both cases. *Bacillus coli* isolated from one, a *Coccus* from the other.⁵ 10 reactions recorded as P₁, 2 as P₂.⁶ Reaction recorded as P₁.⁷ 17 reactions recorded as P₁, 5 as P₂.⁸ 11 reactions recorded as P₁, 3 as P₂.⁹ Reaction recorded as P₁.¹⁰ Reaction recorded as P₁.

In contrast to the above, the cell-solution pullorin detected all of 15 bacteriologically positive birds and produced a reaction in only 1 (1.4 per cent) of 70 bacteriologically negative birds. The size of the pullorin reaction was recorded as P₁ in 8 fowls, and as P₂ in 3. While a more pronounced reaction would have facilitated the readings, it was possible, as the results show, to accurately detect and interpret these reactions.

In addition to the 85 birds just discussed, a flock of 143 fowls, consisting of White and Black Leghorns, Rhode Island Reds, Barred Rocks, Black Minorcas, and representatives of several other breeds, were tested with the cell-solution pullorin and by the agglutination method. These fowls were not available for post-mortem examination

TABLE 6
SUMMARY OF THE RESULTS OF PULLORIN AND AGGLUTINATION TESTS AND OF THE BACTERIOLOGICAL EXAMINATION OF 570 FOWLS

Group	Type of pullorin	Source of pullorin	Bacteriologically positive fowls ¹						Bacteriologically negative fowls ²						
			That were detected by			That were not detected by			That reacted to			Agglutination test			
			Total number of fowls tested	Pullorin test	Agglutination test	Total number of fowls tested	Pullorin test	Agglutination test	Total number of fowls tested	Pullorin test	Agglutination test	Total number of fowls tested	Pullorin test	Agglutination test	
1	Concentrated	Univ. of Calif.	67	9	44.4	9	100	5	55.5	0	0.0	58	8	13.8	
		Commercial	85	70	52	74.2	70	100	18	25.7	0	0.0	15	11	73.3
2	Precipitated	Univ. of Illinois	235	97	88	90.7	96	98.9	9	9.2	1	1.0	138	44	31.8
		Univ. of Calif.	33	2	0	0.0	1	50	2	100.0	1	50.0	31	10	32.2
3	Cell-suspension	Univ. of Calif.	65	14	13	92.8	13	92.8	1	7.1	1	7.1	51	22	43.1
		Univ. of Calif.	85	15	15	100.0	14	93.3	0	0.0	1	6.6	70	1	1.4
4	Cell-solution	Univ. of Calif.	85	15	15	100.0	14	93.3	0	0.0	1	6.6	70	1	1.4
													0	2	2.8

¹ Fowls from which *Bacterium pullorum* was isolated. Gross lesions present in all.

² Fowls from which *Bacterium pullorum* was not isolated.

³ Per cent of bacteriologically positive fowls.

⁴ Per cent of bacteriologically negative fowls.

⁵ These two birds are identical with two of the five bacteriologically negative fowls with gross lesions that reacted to pullorin.

and therefore data concerning them were not included in table 5. All gave a negative reaction to the agglutination test. One fowl, a Black Minorca (C-211), reacted to the pullorin test. The tests on this bird were repeated a month later with the same results. The bird was then secured for autopsy. The only abnormalities found were two small slightly blood-tinged yolks. *Bacterium pullorum* was not isolated. The results of the tests of these 143 negative birds are of value principally in indicating that the intradermal injection of the cell-solution pullorin is not liable to produce a non-specific or false reaction.

SUMMARY AND DISCUSSION

Table 6 gives a summary of the results of all of the pullorin and agglutination tests and of the post-mortem examination of the birds. A study of this table shows a very close correlation between the results of the agglutination tests and the bacteriological findings. A positive agglutination reaction was obtained with all but 4 (1.4 per cent) of the 207 birds from which *Bacterium pullorum* was isolated. This is a surprisingly high percentage of reactions to a single test of a group of infected fowls. Of the 363 bacteriologically negative fowls, all except 9 (2.4 per cent) gave a negative agglutination reaction. This can be regarded as an expected occurrence since the experience of numerous investigators has shown that non-infected fowls seldom react to the agglutination test. The positive agglutination reactions of these bacteriologically negative birds might have been due either to recovery and immunity of the birds, or to infection that was present, but which was not obtained in culture. This latter is particularly apt to be true of the 3 bacteriologically negative birds in which gross lesions were present.

In contrast to the results with the agglutination test, wide discrepancy is seen to exist between the bacteriological findings and the results of the pullorin tests, with the exception of those obtained with the cell-solution pullorin. This discrepancy consists, first, of the failure of from 7.1 per cent to 55.5 per cent of the bacteriologically positive birds in the various groups to react to the pullorin test; and, second, of a positive pullorin reaction in from 13.8 per cent to 73.3 per cent of the bacteriologically negative birds. Variations in these respects are seen in the results obtained from the concentrated, precipitated, and cell-suspension types of pullorin, but, in all instances, the degree of error is sufficient to make the results very unsatisfactory.

The reactions of the 85 birds that were tested with the cell-solution pullorin and later examined bacteriologically, however, are in remarkably close agreement with the bacteriological findings. Furthermore, the results of the test with this pullorin of the 143 birds, only one of which was examined bacteriologically, but all of which failed to react to the agglutination test, furnish evidence that this pullorin is not liable to cause a non-specific or false reaction in non-infected birds. These results, while encouraging, cannot be considered as conclusively indicating the true diagnostic value of the product because of the limited number of fowls on which it has been used.

CONCLUSIONS

The concentrated, alcohol-precipitated, and cell-suspension types of pullorin were not satisfactory preparations for use in the detection of carriers of *Bacterium pullorum*. The agglutination test is so much more accurate for this purpose than intradermal tests performed with these types of pullorin that the latter should be discarded until a better agent for use in making tests by the intradermal method can be perfected. The preliminary results from intradermal injections of the cell-solution type of pullorin indicate that this preparation is a promising agent worthy of further trial.

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A COMPARATIVE INVESTIGATION OF CERTAIN FILM-FORMING FUNGI¹

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Aerobic, film-forming microorganisms of yeast-like appearance occur very commonly on brines used in the storage of olives and vegetables used for pickles and on fermented liquors such as cider, wine, and beer. They are commonly known as *Mycoderma vini* or 'wine flowers' when they occur on fruit products, as *Mycoderma cerevisiae* on cereal products, and as 'film yeast' or 'scum' on pickle brines. These microorganisms are of considerable economic importance, for they bring about certain undesirable conditions in the flavor, odor, and composition of food products on which they grow.

Although certain forms of *Mycoderma cerevisiae* and *Mycoderma vini* have been extensively studied,⁴ the forms occurring on the surface of vegetable and olive brines have received relatively scant attention. The object of this investigation has been to study *Mycodermas*⁵ from these sources in comparison with *Mycoderma vini* and certain molds found in association with this and other *Mycodermas*.

¹ This paper is an extension of a Master's thesis submitted by the senior author.

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⁴ An excellent review of the earlier investigations will be found in reference 1 in the bibliography.

⁵ The *Mycodermas* investigated here are by some writers referred to as 'false yeasts' or *Mycoderma* yeasts.

EXPERIMENTAL PROCEDURE

Certain of the characteristics of twenty-one microorganisms were determined as follows:

Morphological Characteristics.—The macroscopical appearance of pure cultures of the various microorganisms growing in cucumber juice, malt-extract medium, nutrient solutions of sucrose, dextrose lactose, maltose, mannite, and glycerin, cucumber-juice agar and cucumber-juice gelatin was observed.

The size, form, and general microscopical appearance of cells from seven-day old growth on cucumber juice and the microscopic appearance of agar colonies were also observed.

The cultures were tested for spore formation by culturing on moist gypsum blocks.

Cultural Characteristics.—The more important cultural characteristics were studied. These investigations may be classified as follows:

Fermentation Tests.—The rates of fermentation in nutrient solutions of sucrose, dextrose, lactose, maltose, mannite and glycerin were determined by noting the loss in weight of the nutrient solutions at regular intervals, after inoculation with pure cultures of the various microorganisms.

Biochemical Characteristics.—The destruction of added lactic acid in cucumber-juice medium and of natural acids, mostly lactic acid, in dill brine was determined by noting the loss in acid content by titration with sodium hydroxid. The tolerance of the organisms studied to acetic, citric, lactic, malic, oxalic, and tartaric acids was determined by noting the highest concentration of acid at which pellicle formation occurred in synthetic carbohydrate-free medium. The effect of the organisms on these acids was also determined.

The salt tolerance of these organisms was determined by noting the highest concentration of salt at which pellicle formation occurred in various media. The effect of salt on the rate of oxidation of lactic acid in cucumber juice was also observed.

The effect of disturbing the pellicle and of changing the ratio of surface to volume of the nutrient medium on the activity of certain *Mycodermas* was determined by noting the loss in acid content due to oxidation.

The effect of a layer of neutral mineral oil, of pH value of the medium, of oxygen supply, of light and of certain antiseptics and germicides on the formation of the pellicle was determined.

A study was made of the nitrogen fixation by these organisms in a medium low in, but not devoid of, nitrogen was made by determining the nitrogen content of cultures and dilute cucumber juice in which they were grown.

The effect of salt concentration and of pH value of the medium on the death temperatures of certain *Mycodermas* and a *Penicillium* mold was determined.

All experiments were carried out in duplicate, unless otherwise stated in the text, and only pure cultures derived by single-cell isolations from the sources listed elsewhere were used. The cultures used were purified by repeated plating on cucumber juice agar medium. Freshly grown cultures were used for each experiment and the organisms were generally previously cultured in the medium to be used for the particular experiment. This was done to avoid irregularities in behavior due to change of culture media and to secure active cultures.

Preparation of Media.—The various media used in the tests were prepared as follows:

1. Cucumber-juice medium. Fresh cucumbers were crushed and the juice extracted by pressure in a hydraulic press. The juice was boiled in an open kettle with infusorial earth and filtered brilliantly clear. This furnished a medium of amber or straw-yellow color and was found to be suitable for the growth of all the organisms studied. The composition of the medium was varied to suit the nature of the experiment. However, the cucumber juice used in preparing most of the special media was of the following composition:

Balling degree—4.65

Acidity expressed as lactic—0.1 grams per 100 cc

pH (determined electrometrically)—5.7

Cucumber juice was used as the basic culture medium because most of the microorganisms studied were isolated from cucumber-pickle brines. The natural juice and the media made from it were sterilized by auto-claving at 15 pounds pressure for 30 minutes.

2. Dill-pickle brine medium. Brine from fermented dill pickles was boiled in an open kettle with infusorial earth and then filtered clear. The pH value of this medium was 3.2, the density 6° Baumé, and its total acid content expressed as lactic was 1.20 grams per 100 cc. This medium was sterilized by heating in live steam at approximately 100° C for one hour. Later incubation tests proved this method of sterilization satisfactory. This medium was found suitable for the growth of the majority but not all of the microorganisms studied.

3. Malt-extract medium. 'Spramalt,' a dried preparation of malt extract, was dissolved in water and the solution brought to about 15° Balling. It was sterilized by the usual method of discontinuous sterilization in live steam at approximately 100° C. All of the micro-organisms studied grew well in this medium.

4. Synthetic medium. The synthetic nutrient solution used for determining the action of the microorganisms on the various sugars and acids consisted of 0.01 grams of magnesium sulfate, 0.5 grams of dipotassium phosphate and 10 grams of bacto-peptone dissolved in 1,000 cc of distilled water. This solution furnished a satisfactory medium for the tests in which it was used.

The sugar solutions used in the fermentation tests were sterilized by the usual method of discontinuous sterilization at 100° C.

All media were tested for sterility before use and the methods of sterilization used were found adequate.

TABLE 1
SOURCES OF THE MICROORGANISMS STUDIED

Laboratory number of organism	Kind of microorganism and its source
1	<i>Mycoderma</i> from surface of brine from green olives prepared at Visalia, 1925.
2	<i>Mycoderma</i> from surface of brine used for storage of ripe olives at Lindsay, 1926.
3	<i>Mycoderma</i> . Source similar to that of No. 2.
4	<i>Mycoderma</i> from dill pickle brine from H. J. Heinz Co., Berkeley, 1927.
5	<i>Mycoderma</i> from dill pickle brine from Mueller Bros., Oakland, 1927.
6	<i>Mycoderma</i> . Same source as No. 4.
7	<i>Mycoderma</i> from cucumber storage brine ("salt stock" brine), California Conserving Co., Hayward, 1927.
8	<i>Mycoderma</i> from "salt stock" brine, Mueller Brothers Co., Oakland, 1927.
9	<i>Mycoderma</i> from same source as No. 7.
10	<i>Mycoderma</i> from "salt stock" brine. H. J. Heinz Co., Watsonville, 1927.
11	<i>Mycoderma</i> from fermented apple juice (vinegar stock) from H. J. Heinz Co., Watsonville, 1927.
12	<i>Mycoderma</i> from fermenting apple juice, H. J. Heinz Co., Watsonville, 1927.
13	<i>Mycoderma</i> from fermented apple juice from Jones Vinegar Co., Watsonville, 1927.
14	<i>Mycoderma</i> isolated from California grapes in 1912 by W. V. Cruess.
15	<i>Mycoderma</i> . Same source as No. 14.
16	<i>Mycoderma</i> . Same source as No. 14.
17	<i>Torula</i> , pink. From soil in cucumber field near Hayward, 1927.
18	<i>Penicillium</i> mold. Forms a red pigment. From dill-pickle brine from H. J. Heinz Co., Berkeley, 1927.
19	<i>Penicillium</i> mold. Forms brown conidia. From olive-storage brine from Visalia, 1926.
20	<i>Mucor</i> mold from moldy cucumbers.
21	<i>Penicillium</i> mold. Forms dark green conidia. From "salt stock" brine from California Conserving Co., Hayward, 1927.

SOURCES OF MICROORGANISMS STUDIED

Twenty-one organisms were studied. Of these, 16 are *Mycodermas*, isolated from the several different sources given in the following list: one is a pink *Torula* which grows in the form of a ring on liquid media in test tubes; four are molds of which 3 are *Penicillium* species and 1 is a *Mucor*. All of the organisms described in this report were isolated by us from the sources indicated in table 1, which also gives the laboratory number of each.

Since the principal object of this investigation was to compare the principal morphological and cultural characteristics of the various organisms, they will not be described individually but rather collectively and in relation to one another.

The fresh-olive storage brines from which some of the organisms were isolated were of approximately 9° Baumé and contained 0.2–0.5 per cent acid expressed as lactic. The green-olive brines mentioned in the list were from 50-gallon casks of green Manzanillo olives prepared by the Spanish fermentation process. The dill brines were from commercially prepared 50-gallon casks of dill pickles prepared by the usual fermentation process. These brines were of 5–7° Baumé and contained from 1.1 to 1.5 per cent acid expressed as lactic. The salt stock brines were of 16–18° Baumé and contained less than 0.5 per cent acid expressed as lactic at the time of isolation of the organisms. The fermented apple juice from which some of the organisms were isolated was stored in large, partially open vats, and the film growth on the liquid was used for plating. The *Mycoderma* cultures from grapes were obtained by washing the grapes with sterile water and making culture plates from the washings.

MORPHOLOGICAL CHARACTERISTICS

Growth on Liquid Media.—All of the organisms are strongly aerobic and formed films on quiescent culture liquids. In cultures of organisms 1 to 16, a pellicle appeared at the beginning of development, generally white but changing to a light gray in old cultures. The pellicles were not viscous but were quite fragile and very easily broken. Growth took place also along the sides of the tube to a height of more than three centimeters and the upper portions of this growth invariably turned brown. Some sediment was formed by all.

TABLE 2
MACROSCOPICAL APPEARANCE OF ORGANISMS INVESTIGATED

Medium	Organism	Macroscopical appearance
Cucumber juice	1, 2, 4, 5, 6, 8	Fragile but rather thick, wrinkled, chalky white film, spreading upward 2-3 cm on walls of tube, where it in time becomes brown. Medium becomes turbid. Numerous granules form below the film, some of which settle to the bottom of the tube forming a loose, granular sediment. Very little gas.
	3, 7, 13	Thin, only lightly wrinkled film; considerable growth on walls of tube; turbid medium; much fine sediment. Little to no gas.
	9, 10	Fragile, wrinkled, rather thick film; turbid medium; compact sediment. Little to no gas.
	11	Similar to No. 3 except film more wrinkled and heavier.
	12	Appearance similar to No. 1. Forms much gas.
	14	Thick, slightly wrinkled film, slight sediment. Liquid like No. 1. Much gas.
	15	Smooth, slight film growth, heavy sediment. Medium turbid with small suspended granules. Noticeable gas formation.
	16	Appearance similar to No. 15; but less gas.
	17	Principally in the medium; decided ring formation at surface; much sediment. Ring and sediment pink. No gas.
	18	Typical <i>Penicillium</i> mycelium and conidia. In later stages of growth, surface growth dark green to yellow; medium and under portion of growth become deep red. Pigment does not change in color with reaction of medium.
	19	Typical <i>Penicillium</i> mycelium and brown conidia.
	21	Typical <i>Penicillium</i> mycelium and dark green conidia.
	20	Typical, gray, hair-like <i>Mucor</i> mycelium with typical spherical sporangiophores.
Cucumber juice diluted 1 to 3 with water	1 to 17	Similar to growth in undiluted juice.
Diluted malt syrup	18, 19, 20, 21	Growth much less vigorous than in undiluted juice.
Dill-pickle-brine	1, 2, 4, 5, 6, 14	Growth less luxuriant; films thinner; more granular and more gray and less sediment than in cucumber juice.
Dill-pickle-brine	3	Film whiter and growth less vigorous than Nos. 1, 2, 4, 5, and 6.
	7 to 13	Observations similar to but growth less vigorous than Nos. 1, 2, 4, 5, 14.
	19	Growth scant.
Nutrient sucrose solution	9, 17, 18, 20, 21	No growth.
Nutrient dextrose solution	1 to 21	Growth similar to that in cucumber juice, but no visible gas formation; Nos. 4, 5, and 10 grew more luxuriantly than others.
Nutrient lactose solution	1 to 21	Growth similar to that in cucumber juice, but no visible gas formation; Nos. 4, 5, and 10 grew more luxuriantly than others.
Nutrient maltose solution	1 to 17	Growth slow and slight. No gas.
Nutrient maltose solution	18 to 21	Growth fairly vigorous.
Nutrient mannite solution	1 to 21	Growth vigorous and characteristic. No visible gas formation.
	1, 2, 7, 9, 10, 13	Moderate growth. No gas.
	17, 18, 19, 20, 21	Vigorous growth. No gas.
	3, 4, 5, 6, 8, 11,	{ Scant growth. No gas.
	12, 14, 15, 16	{ Faint growth. No gas.
	1, 4, 5, 8, 10, 11,	{ Good growth. No gas.
	13, 15, 16	{ Vigorous growth. No gas.
	2, 3, 6, 7, 9, 12,	
	14, 17	
	18, 19, 20, 21	

They differed among themselves in the general appearance of the film, the amount and character of sediment, and in the appearance of the substrate, whether clouded or clear.

The macroscopical appearance of the microorganisms in the various media studied is shown in table 2.

TABLE 3
MICROSCOPICAL APPEARANCE OF CELLS OF ORGANISMS 1 TO 17

Organism	Microscopical appearance of cells	Size in microns		
		Average	Maximum	Minimum
1	Sausage-shaped to filamentous. Chain formation common.....	2 x 10	2 x 12	3 x 4
2	Spherical to long rod-shape. Sausage-shaped predominates.....	4 x 3	4 x 8	3 x 3
3	Spherical to oval.....	4 x 4	4 x 6	2 x 2
4	Sausage-shaped to filamentous. Branched chains common.....	2 x 8	4 x 3	2 x 4
5	Short sausage-shaped.....	4 x 6	4 x 10	2 x 3
6	Sausage-shaped to filamentous. Tendency to form a mycelium.....	2 x 8	2 x 15	2 x 7
7	Spherical to ellipsoidal; some sausage-shaped.....	3 x 4	2 x 6	2 x 2
8	Spherical to sausage-shaped.....	4 x 5	3 x 8	2 x 2
9	Spherical to short sausage-shaped.....	4 x 4	4 x 8	2 x 2
10	Spherical to short ellipsoidal.....	3 x 3	4 x 4	2 x 2
11	Spherical to sausage-shaped.....	3 x 4	4 x 6	2 x 3
12	Spherical to sausage-shaped. Much variation in size.....	4 x 8	4 x 14	2 x 2
13	Short sausage-shaped cells predominate.....	4 x 6	4 x 8	2 x 3
14	Sausage-shaped. Branched chain growth common.....	4 x 9	4 x 16	4 x 5
15	Spherical to ellipsoidal.....	3 x 5	4 x 6	1.5 x 1.5
16	Spherical to sausage-shaped. Forms branched chains.....	4 x 4	6 x 6	2 x 2
17	Spherical.....	4 x 4	6 x 6	2 x 2

TABLE 4
APPEARANCE OF COLONIES ON SOLID MEDIA

Organism	Growth on agar media	Growth on gelatin media
1, 2, 4	Small, irregular, convex, rugose, cretaceous colonies. Edges entire under microscope.	Small, irregular, flat (but slightly raised), rugose, gray, opaque colonies. Edges entire under microscope.
3	Small, irregular, convex, rugose, opaque colonies. Edges entire under microscope.	Similar to No. 1.
5	Similar to No. 1.	Small, irregular, concave, rugose, gray, opaque colonies. Edges entire under microscope.
6	Similar to No. 3.	Similar to No. 1.
7	Similar to No. 1.	Similar to No. 5.
8	Similar to No. 3, but slightly brown.	Similar to No. 1.
9, 10	Similar to No. 1.	Small, irregular, raised, rugose, cretaceous colonies. Edges entire under microscope.
11, 12, 13, 14,	Similar to No. 1.	Small, punctiform, opaque gray colonies. Edges entire under microscope.
15, 16		
17	Small, irregular, convex to palomate, smooth, glistening, pink colonies. Edges entire under microscope.	Small, round, concave, smooth, glistening pink colonies. Edges entire under microscope.
18, 19, 20, 21	Typical mycelial mold colonies.	Typical mycelial mold colonies.

It is evident from these observations that the organisms differ in their growth on and in various media. Relative vigor of growth among the twenty-one organisms varied considerably according to the medium.

The size, form, and general appearance of organisms 1 to 17 after culture for seven days at room temperature are given in table 3.

Growth on Cucumber-Juice Agar and Gelatin.—The appearance of seven-day-old colonies on cucumber-juice agar and on cucumber-juice gelatin are given according to the terminology adopted by Chester⁽²⁾ in table 4.

Spore Formation.—Using the usual methods of causing spore formation^(1, 3)—by growth in moist gypsum blocks or in saturated gypsum solutions—no spore formation could be observed with cultures 1 to 17, although under the same conditions yeasts which were known to form spores (*Saccharomyces ellipsoideus*) sporulated satisfactorily.

FERMENTATION TESTS

A synthetic nutrient medium was prepared as described on page 204. Sucrose, dextrose, lactose, and maltose were added in concentration of 10 grams per 100 cc, and glycerin and mannite in concentration of 5 grams per 100 cc. Seventy cc portions of sucrose and dextrose were placed in 125 cc Erlenmeyer flasks, 80 cc portions of lactose and maltose and 70 cc portions of glycerin and mannite were placed in 4-ounce bottles.

After sterilization the solutions were inoculated with the different organisms, and loss in weight during the incubation period at room temperature was determined. This loss corrected for loss in weight from evaporation was used as a measure of fermentation. A lower concentration of glycerin was used because of its greater osmotic pressure. The loss in weight of the inoculated flasks after correction for losses by evaporation at the end of 30 days is given in table 5.

An examination of table 5 will show that most of the organisms studied caused slight to moderate destruction of dextrose, sucrose and maltose but with the exception of the molds did not destroy any appreciable proportion of the lactose or mannite. However, organism No. 3 fermented mannite feebly.

The organisms in general grew best in the maltose medium, with dextrose a close second, and sucrose, glycerin, mannite, and lactose in the order stated. They grew very poorly in lactose. At the end of two months the pH value of the solutions given in table 5 was determined electrometrically and the data are shown in table 6.

TABLE 5
LOSS IN WEIGHT IN GRAMS IN 30 DAYS

Organism	Sucrose* grams	Dextrose* grams	Lactose† grams	Maltose† grams	Glycerin‡ grams	Mannite‡ grams
1.....	0.42	2.31	0.00	0.45	0.25	0.00
2.....	0.19	2.40	0.00	0.23	0.50	0.00
3.....	0.18	1.25	0.20	0.50	0.21	1.31
4.....	0.54	3.16	0.15	0.38	0.54	0.00
5.....	0.30	3.08	0.23	0.28	0.38	0.00
6.....	0.26	4.16	0.00	0.50	0.24	0.00
7.....	0.90	1.16	0.14	0.63	0.26	0.00
8.....	0.25	1.61	0.00	0.29	0.60	0.04
9.....	0.71	0.97	0.00	0.50	0.51	0.00
10.....	0.39	0.71	0.00	0.50	0.37	0.00
11.....	0.04	0.57	0.00	0.77	0.09	0.00
12.....	1.11	0.43	0.52	0.20	0.08	0.00
13.....	0.41	0.27	0.77	1.00	0.33	0.00
14.....	0.70	3.75	0.00	0.28	0.32	0.00
15.....	0.20	3.66	0.00	0.19	0.48	0.00
16.....	0.18	0.72	0.00	0.19	0.00	0.00
17.....	0.19	0.43	0.00	0.00	0.44	0.00
18.....	1.16		0.00	1.53	0.40	0.00
19.....	0.60	2.47	1.20	1.43	0.72	0.65
20.....	0.06	3.16	0.00	1.68	0.57	0.02
21.....	1.61	0.64	1.02	1.49	0.35	0.34

* Concentration 10 grams per 100 cc; 70 cc of solution used in 125-cc Erlenmeyer flask.

† Concentration 10 grams per 100 cc; 80 cc of solution used in 4-oz. bottles.

‡ Concentration 5 grams per 100 cc; 70 cc of solution used in 4-oz. bottles.

TABLE 6

CHANGE IN pH VALUE PRODUCED BY GROWTH OF ORGANISMS 1 TO 21 AFTER 60 DAYS

Organism	Sucrose pH	Dextrose pH	Maltose pH	Glycerin pH	Mannite pH
Control.....	5.60	4.90	5.10	6.4	6.8
1.....	5.35	4.00	5.00		
2.....	6.20	4.00	4.75	5.3	6.8
3.....	4.70	3.60	4.50	6.4	6.8
4.....	4.50	3.65	4.10	6.9	8.2
5.....	5.35	3.80	5.10	5.8	6.8
6.....	6.35	4.30	4.80	6.4	7.5
7.....	4.35	4.10	4.75	7.0	7.4
8.....	3.65	3.80	4.80	6.1	6.4
9.....	4.15	4.00	4.35	7.0	7.1
10.....	4.35	4.05	4.50	7.5	7.1
11.....	5.50	2.85	3.60	6.2	7.1
12.....	2.00	2.10	4.30	5.1	7.2
13.....	4.30	2.40	4.40	6.8	7.0
14.....	5.30	5.00	5.10	6.7	6.9
15.....	5.10	4.10	5.40	6.2	7.7
16.....	5.05	3.90	4.70	6.4	6.6
17.....	4.10	3.50	4.40		7.0
18.....	3.20	4.00	3.60	5.5	5.1
19.....	7.00	6.10	4.90		6.7
20.....	3.75	3.15	2.80	3.3	7.3
21.....	6.70	6.15	5.90	6.5	6.6

It had been noted previously by various workers that certain fungi are capable of changing the reaction of the media which they ferment, that is, are capable of producing acids or of destroying them.

It is evident that the majority of these organisms were capable of producing acid especially in sucrose, dextrose, and maltose solutions. The nature of the acid was not determined. Titration of total acid with N/10 sodium hydroxid confirmed pH determinations and the total acidities were found to be in the inverse order of the pH values.

DESTRUCTION OF AND TOLERANCE FOR CERTAIN ORGANIC ACIDS

It has long been known that the *Mycodermas* reduce the acidity of pickle brines by oxidation, as reported in publications such as those of Round and Lang,⁽⁴⁾ Le Fevre,⁽⁵⁾ Shinkle,⁽⁶⁾ Cruess,⁽⁷⁾ and others. It is commonly recommended that the 'scum' forming on brines be skimmed frequently to avoid loss of lactic acid on which the keeping quality of the product depends.

Previous to the preliminary report of Joslyn⁽⁸⁾ no data on the rate of destruction of the acid could be found in the literature. Joslyn found that the mixed culture of *Mycoderma* used by him destroyed acid more rapidly than the pure culture; both, however, destroyed more than half of the acid originally present (1.13 per cent) in one month at 80° F. Destruction was somewhat slower at room temperature than at 80° F. Cruess⁽⁹⁾ determined the rate of destruction of lactic acid in olive brines by three pure cultures of *Mycoderma* a culture of *Penicillium glaucum*, and two mixed cultures. He found that the *Penicillium* mold destroyed lactic acid about as rapidly as the *Mycoderma* cultures.

Lactic Acid in Cucumber Medium.—In our studies the lactic-acid-destroying activity of organisms 1 to 21 in a cucumber medium was determined in the following manner: The organisms were cultured in cucumber juice for one week prior to the test; one cc of the well-shaken culture was pipetted into 150 cc of sterile cucumber juice containing 0.9 gram of added lactic acid per 100 cc. The rate of oxidation of lactic acid was determined by noting the loss in total acid as shown by titration with N/10 sodium hydroxid at regular intervals. The data is given in table 7.

In order to avoid an error from the carbon dioxide formed, especially in cultures 3, 6, 15, and 20, the samples to be titrated were

either heated slightly below the boiling temperature for several minutes or were diluted with boiling distilled water to volatilize carbon dioxide before titration. The pellicle in each culture was unavoidably disturbed upon shaking before removal of the sample. Such disturbance interfered with the activity of the organisms, as will be shown later.

The organisms differed from each other in their rate of oxidation of lactic acid. Organisms 1, 4, 8, 9, 14, 16, and 21 oxidized lactic acid rather rapidly; organisms 2, 7, 10, 13, 19, and 20 not so rapidly; organisms 3, 17, and 18, slowly.

TABLE 7
PERCENTAGE OF LACTIC ACID OXIDIZED

Organism No.	Time in days			
	7 days per cent	14 days per cent	21 days per cent	35 days per cent
1	31.9	49.0	72.9
2	30.9	42.3	67.2
3				38.5
4	22.4	28.8	53.8	78.8
5	28.5	38.4	81.7
6	0	33.6	52.0	71.1
7	14.0	25.0	29.8	60.6
8	26.5	38.5	48.0	88.5
9	18.8	42.2	44.2	76.8
10	9.7	11.5	32.7	63.5
13	18.7	29.8	62.5
14	9.2	38.5	51.9	80.7
15		15.4	23.1	71.2
16	18.8	32.7	51.9	74.0
17	3.4	3.8	7.7	35.7
18	13.1	32.7	43.0
19	23.7	49.0	59.6
20		13.5	19.3	66.3
21	25.1	28.8	38.5	74.0

It would have been desirable to note whether or not the *Mycodermas* were capable of oxidizing all the lactic acid and producing an alkaline reaction in the medium. Unfortunately in the later stages of the experiment, cultures 1 to 17 inclusive became contaminated, chiefly with mold. However, the cultures were retained for three months. Molds 19, 20, and 21 had produced an alkaline reaction in the medium; of the infected *Mycoderm*a cultures, only Nos. 16 and 17 developed an alkaline reaction. In experiments reported by Cruess⁽⁹⁾ only one culture (a mixture of *Mycoderm*a and mold) produced an alkaline reaction in 7 months' incubation of inoculated olive brines.

In Dill Brine.—We also determined the rate of oxidation by these organisms of the acid naturally formed in dill-pickle brine in the following manner. Ten-cubic-centimeter portions of dill brine were placed in plugged test tubes. These were sterilized and then inoculated with loopfuls of organisms 1 to 21. A sufficient number of test tubes was prepared so that at each examination an undisturbed 10 cc sample of each could be used for titration.

Organisms 17, 18, 20, and 21 failed to grow in the dill brine. The rate of oxidation of the natural acid in the dill brine is shown in table 8.

TABLE 8
PERCENTAGE OF ACID OXIDIZED IN DILL BRINE

Organism	Time in days					
	8 days per cent	13 days per cent	18 days per cent	23 days per cent	39 days per cent	81 days per cent
1.....	53.3	71.7	92.3	96.2	98.5	99.0
2.....	53.7	71.5	91.0	94.0	97.0	97.5
3.....	15.0	42.4	54.5	60.6	95.5	97.5
4.....	54.5	82.5	92.5	94.0	98.5	98.5
5.....	55.3	75.8	89.6	94.7	97.0	97.5
6.....	59.0	72.0	89.4	96.2	98.5	99.0
7.....	34.8	74.2	91.0	98.5	Basic	Basic
8.....	43.2	57.6	85.0	91.0	97.0	97.0
9.....	18.2	50.8	79.5	96.2	Basic	Basic
10.....	49.2	82.0	91.0	98.0	Basic	Basic
11.....	50.0	75.0	77.2	79.5	82.6	91.0
12.....	61.3	77.3	94.0	97.0	98.5	98.5
13.....	30.3	47.0	73.4	77.3	80.2	80.2
14.....	62.8	83.5	96.2	97.5	99.3	99.3
15.....	50.0	54.5	76.0	94.0	98.5	99.5
16.....	22.7	34.8	43.2	47.0	60.6	91.5
19.....	18.2	59.0	89.5	97.8	Basic	Basic

The organisms did not differ as much from each other in their rate of oxidation of acid in this test as they did in cucumber-juice medium, but the loss of acid is more rapid. Organisms 1, 2, 4, 5, 6, 11, 12, 14, and 15 exhibited similar rates of oxidation of acid. The initial rate was rapid but decreased with decrease in concentration of acid. Organisms 3, 7, 8, 9, 10, 16, and 19 oxidized the acid slowly at first but more rapidly as the concentration of the acid decreased. Organism 13 oxidized the acid least rapidly. Organisms 7, 9, 10, and 19 produced an alkaline reaction in the dill brine. Typical rates of acid-destruction are shown by the curves in figure 1.

In both the cucumber juice and the dill-pickle brine, darkening of the medium occurred when low total acid was reached. This dark color persisted even after acidifying the medium. It may have been caused by oxidation of some coloring material of the brine.

Destruction of Various Organic Acids in a Sugar-Free Medium.—It is evident that these organisms were able to destroy lactic acid. However, sugars and other carbon compounds were present in the above media and these may have furnished some of the carbon and energy for growth.

In order to eliminate sugars, nutrient-acid solutions were prepared by adding to the sugar-free nutrient media previously described

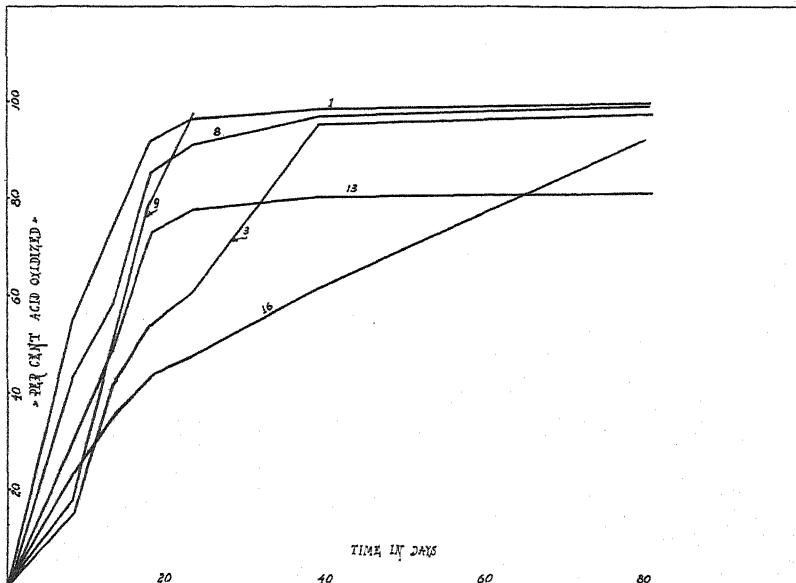


Fig. 1. Rate of oxidation of acid in dill brine.

(page 204) various amounts of acetic acid, citric acid, lactic acid, malic acid, oxalic acid, and tartaric acid. The sterile, tubed acid media were inoculated with loopfuls of the organisms which had been grown for two weeks in malt extract medium. These tubes were then stored at room temperature and growth or its absence in each tube determined periodically. It was found that film formation occurred sooner in the media containing the smaller amounts of acid and sooner in solutions of citric, lactic, and malic acids than in those of the other acids. The examinations were continued over a period of from 3 to 4 weeks incubation. The results were as follows:

In acetic acid, organisms 1 to 21 inclusive grew in 0.024 N (0.14 per cent) solution, all except Nos. 18, 20, and 21 grew in 0.042 N (0.25 per cent) solution; all except Nos. 7, 9, 10, 17, 18, 20, and 21 grew in 0.060 N (0.36 per cent), 0.76 N (0.46 per cent and pH of 4.5),

and 0.095 N (0.57 per cent); all except the aforementioned and No. 19 grew in 0.140 N (0.84 per cent and pH of 3.8); only Nos 1, 2, 3, and 11 grew in 0.28 N (1.68 per cent, pH 3.5) and none grew in 0.67 N (4.0 per cent pH 3.35).

In citric acid all except Nos. 7 and 9 grew in the solution of 0.2 N (1.28 per cent pH 2.75); all except Nos. 4, 7, 9, and 10 in 0.38 N (2.43 per cent, pH 2.5); and in 0.6 N (3.84 per cent, pH 2.3) all except Nos. 4, 7, 9, 10, 11, and 19; in 0.82 N (5.25 per cent pH 2.1); and in 1.0 N (6.4 per cent, pH 1.9) only Nos. 6, 12, 14, 15, 16, 17, and 18 grew.

In malic acid Nos. 7, 9, 10, 13, and 16 failed to grow at 0.2 N (1.34 per cent, pH 2.65); Nos. 7, 9, 10, 11, 13, 16, 19, 20, and 21 failed to grow at 0.42 N (2.81 per cent, pH 2.3); Nos. 7, 8, 9, 10, 11, 13, 16, 17, 19, 20, and 21 failed to grow at 0.62 N (4.15 per cent, pH 2.1); Nos. 5, 7 to 13 inclusive, 15, 16, 17, 19, 20, and 21 failed to grow at 0.95 N (6.36 per cent, pH 1.85); and only Nos. 2, 6, and 18 grew in 1.02 N (6.7 per cent, pH 1.8).

In oxalic acid Nos. 3, 11, and 13 failed to grow at 0.016 N (0.072 per cent); Nos. 3, 11, 13, and 16 at 0.027 N (0.122 per cent), and Nos. 1 to 5 inclusive, 7 to 11 inclusive, 13, and 16 failed to grow at 0.06 N (0.27 per cent).

In tartaric acid Nos. 1, 4, 5, and 7 to 11 inclusive, and 13 did not grow at 0.2 N (1.50 per cent, pH 2.6); Nos. 1, 2, 4, 5, 7 to 11 inclusive, 13, 15, 16, and 17 failed to grow at 0.37 N (2.78 per cent, pH 2.35); Nos. 1, 2, 4 to 13 inclusive, 15, 16, 17, and 20 failed to grow at 0.55 N (4.13 per cent, pH 2.10); Nos. 1, 2, 3 to 13 inclusive, 15, 16, 17, 19, 20, and 21 failed to grow at 0.77 N (5.78 per cent, pH 1.95), and also at 0.94 N (7.05 per cent, pH 1.85).

Most of the organisms studied were able to utilize the acids investigated for growth when the concentration was not too high. Of the acids investigated lactic acid was more suitable to the growth of yeast and citric acid to the growth of molds. Reference to figure 2 will show that the acids studied can be arranged as follows in order of their suitability for the growth of organisms studied at the concentrations investigated: citric, lactic, malic, tartaric, acetic, and oxalic. The organisms studied had a lower tolerance for oxalic acid than for any of the other acids studied. Of the molds studied No. 18 was most tolerant of acid. Of the *Mycodermas* Nos. 1, 2, 3, 4, 5, 6, 12, and 14 were most tolerant. While there appeared to be considerable correlation between growth and pH value for lactic, tartaric, and malic acids,

yet these acids apparently also exerted a considerable specific effect; that is, at equal pH values the acids varied in toxicity. For acetic and oxalic acid the specific action was particularly marked.

The culture media were titrated at the end of the incubation period and it was found that invariably reduction in the total acid content accompanied growth. This loss in acid was less in the more acid solutions owing probably to the fact that the amount of growth was less. It is remarkable that the organisms studied were able to obtain food

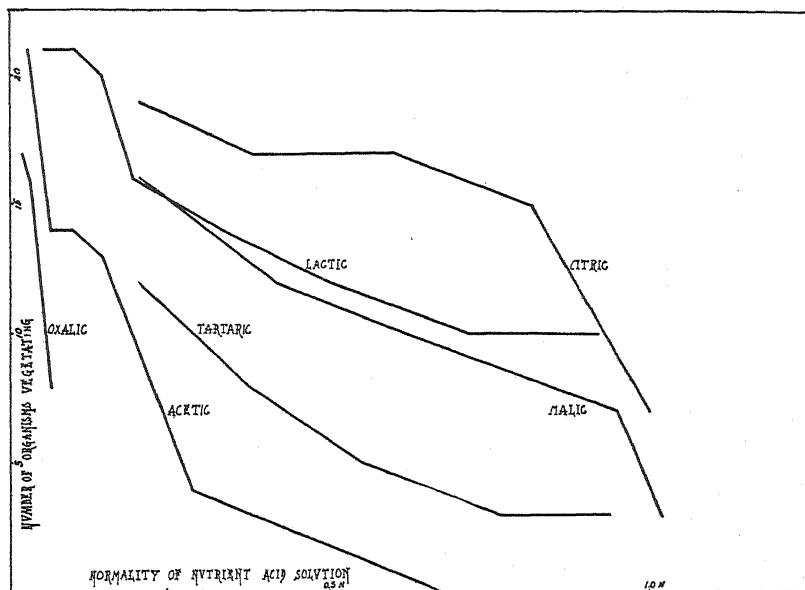


Fig. 2. Acid tolerance in synthetic nutrient medium.

and energy for growth from the oxidation of various organic acids which in the concentrations employed are normally toxic to culture yeasts such as *Saccharomyces ellipsoideus*.

Tolerance for Acids in Brines.—The effect of salt on the acid tolerance of a few of the organisms under investigation was studied. Cucumber juice was diluted 1:3 with distilled water and 10 per cent of sodium chloride was added. To this medium various amounts of acetic, citric, and lactic acids were added. Flasks of the medium were then inoculated with loopfuls of organisms 3, 6, 7, 10, 18, and 20, which were previously cultured in the diluted cucumber-juice medium. The inoculated bottles were stored at room temperature and at the end of 30 days were examined for growth and titrated for acid. At this

time solutions in which no growth was evident were re-inoculated with 1 cc portions of cultures of the organisms mentioned above and incubated for another month. The results obtained are shown in table 9.

The addition of as little as 0.5 per cent acetic acid inhibited the growth of all the organisms in the presence of 10 per cent salt. All of the acids in the concentrations used inhibited the growth of organism No. 20. Organism 18 was especially tolerant of citric acid and fairly tolerant of lactic acid. However, its growth was slight in acid concentrations higher than 1.0 per cent. It grew better than the other organisms in the citric-acid brine. In lactic-acid brines organism 6 grew best with organisms 15 a close second.

TABLE 9
GROWTH IN ACIDIFIED BRINES

Organism No.	No acid	Per cent acetic acid		Per cent citric acid					Per cent lactic acid				
		0.5	1.0	1	2	4	5	6	7	1	2	4	5
3	+			+						+	+		
6	+			+	+	+				+	+	+	
7	+			+						+	+		
10	+			+	+					+	+		
18	+			+	+	+	+	+		+	+		
20	+												

The acid in each culture was titrated at the close of the experiment. In the 1 per cent citric-acid brine the greatest loss of acid was caused by organism 18 and the least by organism 3 and 6. Organisms 7 and 10 caused but little loss in acid. In solutions of higher citric-acid content the loss in acid was but slight, even with organism 18.

In the 1 per cent lactic-acid brine, organisms 7 and 10 caused the greatest loss, organisms 3 and 18 were next, and organism 6 last. The loss in acid in 2 per cent lactic-acid solutions was less but considerable, and in the 4 per cent lactic acid still less, the loss caused by organism 6 being slight.

The presence of 10 per cent salt greatly decreased the tolerance of these organisms for acid; as shown both by growth and by destruction of the acid.

Tolerance for Acids in Fermented Cider.—Freshly fermented cider was sterilized in 4-ounce bottles and was then inoculated with the cultures the organisms under investigation. The inoculated bottles

were stored at room temperature for a period of two months. The cider after sterilization contained 4 per cent alcohol and 0.58 per cent acid as malic.

Of the twenty-one organisms under investigation, only Nos. 1, 2, 3, 5, 6, 8, 11, 12, 13, 14, 15, and 16 grew in the above medium. Organisms 4, 7, 9, 10, 17, 18, 19, 20, and 21 failed to grow in the cider. This would indicate a marked variation in the ability of different *Mycoderma* to utilize natural media for growth. The former group of organisms did not grow luxuriantly in the fermented cider. The addition of 0.5 per cent acetic acid to the fermented cider medium inhibited the growth of only organism 13, but the addition of 1 per cent acetic acid prevented the growth of all organisms. When 2 per cent malic acid was added, the growth of all organisms but Nos. 11, 12, 13, 14 was inhibited, when 3 per cent malic acid was added the growth of all but No. 11 was inhibited, and the addition of 4 per cent malic acid prevented all growth. A lower concentration of acetic acid than of malic acid was necessary to inhibit growth. That the addition of acetic acid will prevent the development of *Mycoderma* in fermented cider has been known previously. (7, 10, 11)

EFFECT OF SODIUM CHLORID

Very little research has been reported in the literature upon the salt tolerance of *Mycodermas* and molds. In studying the conservation of meat and fish by salt, Pettersson⁽¹²⁾ has found that certain wild yeasts can develop in fish in 25 per cent of salt. Bitting⁽¹³⁾ found that 5 grams of salt per 100 cc had no effect on molds in meat bouillon while 30 grams per 100 cc prevented the mold. The yeasts studied by him did not develop in a solution containing 15 grams per 100 cc. Thom⁽¹⁴⁾ found that 15 per cent salt inhibited the growth of certain *Penicillium* molds. Karaffa-Korbut⁽¹⁵⁾ found salt to possess a weak bactericidal power and found that many yeasts were capable of growing in a 25 per cent solution. Mitra⁽¹⁶⁾ found the concentration of salts that inhibit growth of *Saccharomyces ellipsoideus* cells to be 2.2 M potassium chlorid, 1.2 M magnesium chlorid, 0.7 M calcium chlorid and 0.2 M sodium chlorid in a synthetic medium.

Cruess⁽⁹⁾ found that the addition of salt reduced the rate of destruction of added lactic acid by the *Mycodermas* and mold studied by him. Several cultures, including a mold, grew at 16 per cent salt and caused considerable decrease in acidity. One was inhibited at 16 per cent salt but grew slightly at 14 per cent salt. None of the

organisms grew at 18 per cent salt. However, a mixed culture of *Mycoderma* and mold from cucumber brine grew to some extent at 18 per cent salt.

Recently Spearman, Gee, and Luck⁽¹⁷⁾ investigated the influence of sodium chlorid on the growth and metabolism of *Saccharomyces cerevisiae* in a wort medium. They found that *S. cerevisiae* will ferment wort containing up to 10 per cent salt, but the weight of the yeast crop obtained decreased as the salt concentration increased and the lag phase of the fermentation period was progressively lengthened by increasing concentrations of salt.

In our experiments the effect of salt on the growth and metabolism of cultures 1 to 21 was studied. In a preliminary test flasks of sterile cucumber juice to which various amounts of salt had been added were inoculated with 1 cc portions of cultures 1 to 21. Before inoculation the medium used contained 9 grams lactic acid per 100 cc and was of 3.6 pH value. It was found later the pH value of the medium has a marked influence on the salt tolerance of these organisms.

The addition of 4.6 per cent salt had little influence on the rate of oxidation of the lactic acid except by organisms 7 and 20, whose activity was much retarded. The addition of 8.2 per cent salt only slightly retarded the action of organisms 1, 2, 3, 4, 5, and 8, but considerably retarded that of the other organisms. Only organisms 7, 9, and 10 showed growth at the end of 35 days in the 12.5 per cent salt solution. During the 35-day incubation period acid titrations were made at regular intervals. Curves showing the rates of destruction for several organisms are given in figure 3. The retarding effect of the salt is very evident.

The pellicle formed at about equal rates in the natural medium and in that containing 4.6 per cent salt but much more slowly in that containing 8.2 per cent salt and still more slowly in that of 12.5 per cent salt. In this experiment salt exerted a greater retarding effect on the rate of growth of the molds than of the *Mycodermas*.

The activity of the *Mycodermas* as measured by the reduction in total acidity was found to be proportional, within the experimental error, to the amount of growth as determined by centrifuging a known volume of the solution and noting the volume of sediment. It would appear that the amount of acid oxidized by a given volume of the *Mycoderma* in a given time is fairly constant and reduction brought about in the rate of oxidation of lactic acid by the addition of salt is caused by decrease in the size of the *Mycoderma* crop and not in its activity.

It was noted that the addition of moderate amounts of salt, e.g., 2 to 4 per cent, increased the *Mycoderma* crop of some cultures and the amount of lactic acid oxidized in a period of 30 days. However, this evidence of growth stimulation was not consistent and not very conclusive.

Salt Concentration Required to Inhibit Growth.—In one experiment a cucumber-juice medium was brought to pH 3.6 by the addition of lactic acid and the following percentages of salt were added

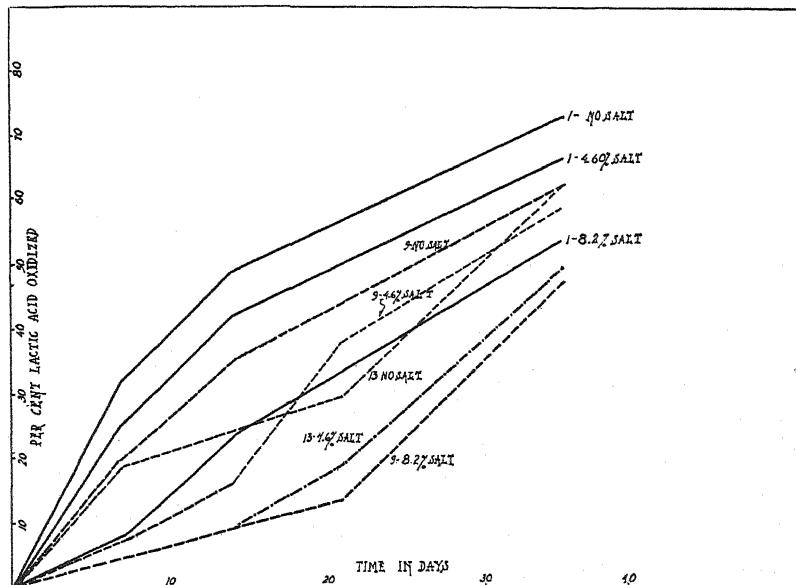


Fig. 3. Effect of salt on the rate of oxidation of lactic acid.

and checked by titration with silver nitrate: 0, 4.6, 8.2, 12.5, and 15 per cent. After sterilization, inoculation, and 30 days' incubation it was found that all cultures had grown in the media of 0, and 4.6 per cent salt; all except No. 13 had grown in 8.2 per cent salt; only Nos. 7, 9, 10, 12, and 21 had grown in 12.5 per cent salt and none had grown in 15 per cent salt.

In dill brine the tolerance of the organisms for salt was somewhat less than in cucumber juice, probably because the dill brine was much poorer in nutrient substances and was of somewhat lower pH value, namely 3.2. All grew in brine of 6° Baumé; all except No. 13 grew in brine of 9.5° Baumé; Nos. 1, 2, 4, 5, and 8 grew in brine of 12° Baumé; Nos. 1, 2, 4 and 8 grew in brine of 14° Baumé; only No. 4 grew in brine of 16° Baumé and none grew at 18° Baumé. The percentage of salt was somewhat less than the Baumé degree.

The tolerance for salt in a cucumber-juice medium of pH 5.10 was found to be greater than the tolerance in dill brine or in acidified cucumber juice. Organisms 1 to 21 previously cultured in plain cucumber juice were transferred to tubes of sterile cucumber juice containing 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 per cent added salt. After six weeks' incubation it was found that the minimum amount of salt necessary to inhibit growth under these conditions was as follows: The addition of 8 per cent salt inhibited the growth of only organism No. 13; 10 per cent salt inhibited growth of Nos. 11, 12, and 16; 12 per cent salt inhibited growth of Nos. 3, 6, 14, and 15; 14 per cent salt was necessary to inhibit growth of Nos. 1, 2, 4, 5, 8, 17, and 20; 20 per cent salt for 7, 9, 10, and 19; and growth of organism 21 occurred in the 20 per cent salt solution.

It was thought the tolerance of the film yeasts for salt might be affected by the salt content of the medium in which they had previously been cultured. Accordingly several of the film yeasts were cultured in cucumber juice containing no added salt and in that containing 10 per cent added salt. Transfers were made to tubes of sterile juice containing 2, 4, 6, 8, 10, 12, 14, 16, and 18 per cent added salt.

After six weeks incubation it was found that most of the cultures previously grown in a medium containing 10 per cent salt possessed a tolerance for 2 per cent more salt than those previously grown on a medium containing no added salt. For examples, organisms, 1, 2, 5, and 8, previously grown in the absence of salt tolerated 12 per cent salt; those previously grown in 10 per cent salt tolerated 14 per cent salt; organism 3 previously grown in the absence of salt tolerated only 10 per cent salt; that grown in 10 per cent salt tolerated 16 per cent salt. Organisms 12, 14, and 15 tolerated 2 per cent more salt when previously grown in 10 per cent brine. Cultures from dill brine (which contains 4 to 5 per cent salt) possessed greater tolerance for salt than those from plain cucumber juice.

The effect of the pH value of the medium was emphasized by the foregoing experiment. The pH value of the medium in that experiment was 5.6, while another similar experiment the pH value was 3.6. At pH 5.6 the tolerance of the organisms for salt was much greater than at 3.6. Thus at pH 3.6 organisms 7, 9, 10, and 19 tolerated 12.5 per cent salt but not 15 per cent, whereas in a medium of pH 5.6 they tolerated 18 per cent salt. Organism No. 21 tolerated 12.5 per cent salt at pH 3.6 and 20 per cent at pH 5.6. In another experiment organisms 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 17, 18, 19, and 20 grew in cucumber

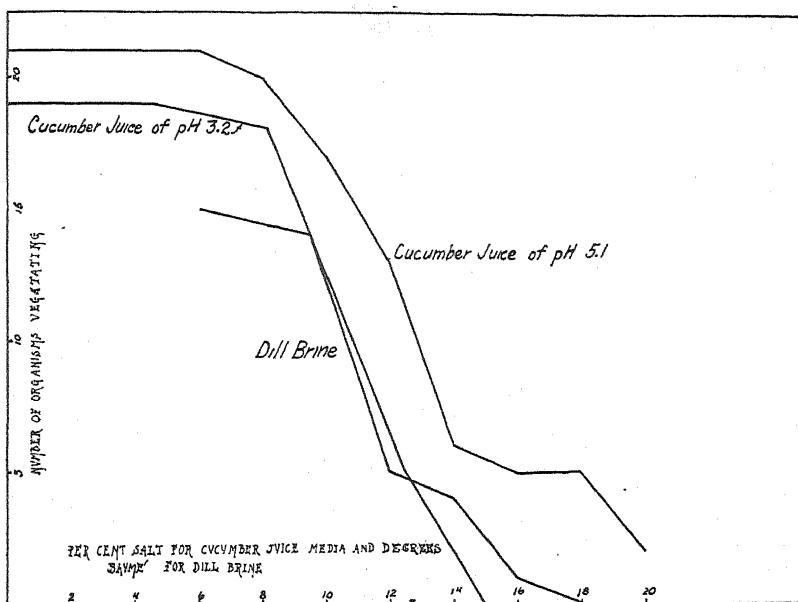


Fig. 4. Salt tolerance in cucumber juice and dill brine.

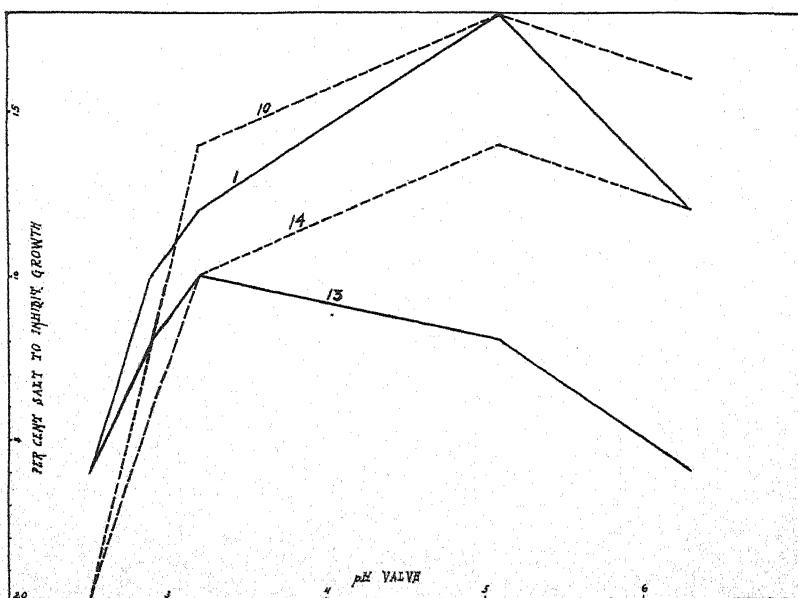


Fig. 5. Effect of pH on salt tolerance.

juice containing 10 per cent salt while only No. 20 grew in the same medium acidified with 1.5 per cent lactic acid. In order to test the effect of pH value on salt tolerance more accurately seven of the organisms were cultured in cucumber-juice media of pH 2.5, 2.9, 3.2, 5.1, and 6.3 to which various concentrations of salt were added. The results of this series of experiments are summarized in table 10.

TABLE 10
EFFECT OF pH VALUE ON SALT TOLERANCE

Organism	pH				
	2.5	2.9	3.2	5.1	6.3
Per cent salt required to inhibit growth					
1	4	10	12	18	12
6	6	10	12	14	12
10	0	8	14	18	16
13	4	8	10	8	4
14	4	10	12	14	12
17	0	6	10	14	12
21	0	4	10	18	16

The data clearly show that the lower the pH, the less the amount of salt necessary to inhibit growth. However, the maximum amount of salt necessary to inhibit growth naturally varied with the organism.

The salt tolerance of a few of the organisms studied was found to be less in apple cider than in cucumber juice. However, this difference probably was caused not only by the difference in pH value but also by differences in the general composition of the two media.

As previously noted for other experiments, it was noted that the volume of visible growth decreased with increase in salt concentration. This effect was not marked in solutions of low salt content but became very noticeable in solutions of higher salt concentration. In all these tests distinct pellicle formation was taken as the criterion for growth. The results of the tests are summarized in figures 4 and 5. In figure 4, the number of organisms showing growth out of the total of twenty-one organisms studied is plotted against the percentage of salt for cucumber juices of pH 3.2 and pH 5.1 and against the Baumé degree for dill brine. In figure 5 the percentage of salt required to prevent the growth of five organisms is plotted against pH value.

EFFECT OF DISTURBING THE FILM ON GROWTH AND RATE OF ACID DESTRUCTION

In determining the rate of oxidation of lactic acid in a previous test, it was necessary to thoroughly mix the contents of the culture flask in order to secure a representative sample. It was suspected then that this periodic disturbance of the film might influence the true rate of oxidation of lactic acid. To test this assumption the following experiment was conducted:

Eight portions of 70 cc each of sterile dill brine in 4-ounce bottles were inoculated with loopfuls of organism 10. One set of bottles was left undisturbed at room temperature. Another set was shaken two or three times daily. After a period of three weeks the remaining acid

TABLE 11
EFFECT OF DISTURBING FILM ON OXIDATION OF ACID
(Per cent of acid destroyed)

Organism No.	2	10
Undisturbed.....	97.0	94.0
Shaken several times a day.....	26.2	2.5
Aerated.....	97.5	65.5

was determined by titration. In the undisturbed set, 99 per cent of the acid had been destroyed, while in the shaken set only 18.2 per cent of the total acid had been destroyed.

Growth was much heavier in the undisturbed bottles. The periodic shaking did not altogether prevent the appearance of film growth, although it prevented its normal development.

However, in these experiments the factor of aeration was not controlled. That aeration markedly increases the multiplication of yeasts, probably by removing carbon dioxide, has been known for many years. Recently Devereux and Tanner⁽¹⁸⁾ have reviewed the previous work and have found that the growth of the yeasts investigated by them in their synthetic nutrient medium was markedly increased by aeration. They found the effect of aeration to be most marked with *Pichia farinosus*. The maximum unaerated and aerated counts for this organism were respectively 24.0 and 320.0 millions per cubic centimeter. As this yeast was the only one which formed a pellicle, they thought that there might be some correlation between pellicle-forming yeasts and those which respond positively to aeration. This point was not investigated further by them.

In a second experiment the ability of a *Mycoderma* to destroy lactic acid was studied in the absence of film formation when supplied with air by passage of a continuous stream of air through the culture. Air was filtered through five inches of cotton and bubbled through 70 cc portions of dill brine in 125 cc Erlenmeyer flasks, inoculated previously with 1 cc of cultures of organisms 2 and 10. The inoculated medium, however, frothed badly and some growth occurred along the sides of the flask, above the liquid. However, the experiment showed that *Mycodermas* were capable of growing in the bottom of a flask of liquid medium when sufficient air was supplied.

For comparison the effect of disturbing the film by intermittent shaking at infrequent intervals was again determined, and the loss in acid determined at the end of 10 days. The liquids in the aerated flasks were brought to volume before titration to compensate for loss from evaporation. The results obtained are shown in table 11.

Active oxidation of the acid occurred in the continuously aerated liquids, although film formation was prevented.

EFFECT OF RATIO OF SURFACE OF MEDIUM TO VOLUME ON RATE OF DESTRUCTION OF ACID

The changes in the nutrient medium caused by the growth of film-forming organisms are normally, in the absence of aeration, most profound at the surface of the medium where growth takes place. Increasing the surface exposed per unit volume should increase the amount of acid destroyed in a given time. If this were strictly so, then the ratio of the percentage of acid oxidized to the surface exposed per unit volume of the nutrient solution would be constant. To test this hypothesis the following experiment was conducted:

To synthetic nutrient medium previously described was added lactic acid at the rate of 25 cc to 500 cc of solution. Ten, 20, 30, 40,

TABLE 12
EFFECT OF RATIO OF SURFACE TO VOLUME ON AMOUNT OF ACID OXIDIZED

Volume added cc	Height cm	Surface/volume	Acid oxidized per cent	K*
10	4.6	0.217	36.5	168
20	9.1	0.110	19.2	171
30	13.4	0.075	12.8	170
40	17.7	0.057	9.7	171
50	22.3	0.045	7.7	171
60	26.5	0.038	6.5

* Ratio of percentage acid oxidized to surface/volume.

and 50 cc of the acidified medium were placed in flat-bottomed Nessler tubes of approximately uniform bore, 1.7 cm in diameter and sterilized. The tubes were inoculated with organism 10 and the decrease in acid content determined after incubating for ten days at room temperature. The results are shown in table 12.

It is evident that there is very good agreement for the values for K (ratio of percentage of acid oxidized to the surface exposed per unit volume) in this case. This proves that the oxidation of lactic acid occurs mainly at the surface. In preliminary tests in which oil-sample bottles were used and the surface per unit volume varied from 0.08 to 0.80, less close agreement for the value of K was observed, probably because of the greater experimental error involved. But even in these tests, the agreement was such as to show definitely that the rate of oxidation of lactic acid varies in proportion to the surface exposed per unit volume.

EFFECT OF A LAYER OF NEUTRAL OIL ON GROWTH

In some vinegar and pickle factories the vinegar stock or pickle brine in the storage vats is covered with a layer of neutral mineral oil in order to prevent the growth of *Mycoderma*. The oil layer retards or, if thick enough, prevents growth of *Mycoderma* by retarding diffusion of oxygen into the liquid beneath. It was thought desirable to determine the thickness of such an oil layer necessary to prevent growth. Accordingly the following experiments were conducted:

TABLE 13
EFFECT OF THICKNESS OF OIL LAYER ON PERCENTAGE OF ACID OXIDIZED

Thickness of oil film	Organism No.	
	10	21
	Acid oxidized	
mm	per cent	per cent
0	Basic	Basic
0.5	Basic	91.5
1.0	100.0	81.0
1.5	55.5	62.2
2.0	24.0	44.6
3.0	3.8	15.5
6.0	0	0
8.0	0	0
11.0	0	0
17.0	0	0
26.0	0	0

To 50 cc portions of dill brine in 4-ounce bottles enough neutral mineral oil was added to give oil films of the following thicknesses: 0.5, 1, 1.5, 2, 3, 6, 8, 11, 17, and 26 mm. One set of these bottles in duplicate was inoculated with 1 cc portions of a culture of organism 10 and another set with 1 cc portions of a culture of organism 21. The percentages of acid oxidized in 10 days are shown in table 13.

Although the presence of 6 mm of oil prevented decrease in acidity, slight growth of the mold and the yeast took place just beneath the oil film. A very faint growth also occurred where films thicker than 6 mm were used. This slight growth may have been made possible by the small amount of air which gained entrance during inoculation.

EFFECT OF pH VALUE UPON GROWTH

It has been shown earlier in this paper that the pH value of the medium has a marked influence on the salt tolerance of these organisms and that dill brine will not support the growth of all of the organisms studied. This latter condition was thought to be due partly to the relatively low pH value of the medium. In order to obtain further data on the effect of pH value on the growth of film organisms, dill brine was brought to pH values of 2.35, 2.6, 2.85, 2.90, 3.2, 5.6, 6.7, and 9.8, by the addition of lactic acid or of sodium hydroxid. The flasks of the sterilized brine were then inoculated with loopfuls of organisms 1 to 21 and examined for growth after a period of thirty days. That the pH value of the medium is of importance can be seen from the following summary.

None of the organisms grew at pH 2.35 and 9.8; Nos. 1, 2, 4, 5, 6, 8, and 11 to 15 grew at pH 2.6; these, and also Nos. 16 and 19 grew at pH 2.85 and 2.90; all except Nos. 17, 18, 20, and 21 grew at pH 3.2; all grew at pH 5.6; and only Nos. 7, 9, 10, 17, and 21 grew at pH 6.7.

NITROGEN FIXATION

Growth of *Mycoderma* occurs on the surface of 'salt stock' tanks for a period of two years or more even when the film is skimmed at frequent intervals. The fact that this recurring growth can take place in a medium naturally so poor in nitrogen might indicate that nitrogen fixation occurs. Analysis showed the scum yeast to be but little poorer in nitrogen content than brewer's yeast grown in a medium rich in nitrogen.

Lipman⁽¹⁸⁾ has shown that *Mycoderma vini* is capable of fixing nitrogen in media very low in nitrogen.

Using the technique devised by Lipman⁽¹⁹⁾ for his experiments, cultures of the twenty-one organisms were grown for two months in cucumber juice containing only 0.01 per cent nitrogen. Total nitrogen was then determined by analyzing the entire individual cultures. It was found that under the conditions of this test, no nitrogen or at most only a negligible amount was fixed. Evidently these organisms can thrive in a medium low in nitrogen.

EFFECT OF OXYGEN SUPPLY

As stated earlier in this paper the organisms studied were found to be strongly aerobic. Growth did not occur in agar plates stored in an evacuated desiccator, and in slab cultures in agar and gelatin, growth was confined to the surface. As shown elsewhere growth and activity were retarded by thin layers of mineral oil and in some cases entirely inhibited by thick-layers of this soil.

To further determine the effect of size of headspace on growth and activity in a sealed container 8-ounce bottles were completely filled with dill brine lightly inoculated with a mixed *Mycoderma* culture, and then 5, 10, 20, 50, and 100 cc respectively were withdrawn and the bottles sealed with crown caps. After incubating for one month at room temperature the bottles were examined for growth and the loss in acid was determined. It was found that only in the cotton-plugged controls was growth heavy and the decrease in acidity marked. In the sealed bottles a slight pellicle formation was observed in the bottle with 100 cc head space. Surface growth did not occur in any of the other bottles. No significant differences in volume of sediment were obtained by centrifuging 20 cc portions of the cultures. The difference in the acid content was also very slight.

This test was repeated using non-acidified cucumber juice, a more favorable medium, in 4-ounce bottles. Headspaces of 0, 5, 10, 20, 25, 50, 75, and 100 cc were provided as previously described. These bottles were heavily inoculated with a mixed *Mycoderma* culture, sealed with crown caps and incubated. After 24 hours a slight film growth occurred in all except that with 0.0 cc headspace. The pellicle in the bottle with 5 cc headspace was very slight and when disturbed did not re-occur. After seven days' incubation the relative amount of film growth was in proportion to the oxygen supply, being very heavy in the bottles plugged with cotton; somewhat less but still heavy at 100 cc headspace; moderate at 50 and 75 cc headspace; light at 25, 20, and 15 cc; very light at 10 cc and negative at 5 cc headspace.

When the bottles were shaken, the turbidity of the liquid was much greater in the bottles plugged with cotton than in any of the sealed bottles. The amount of growth as determined roughly by volume of sediment and by counting by microscope was in proportion to the volume of headspace.

These observations corroborate the recent investigations of Ayers, Barnby, and Voight.⁽²⁰⁾

EFFECT OF CERTAIN ANTISEPTICS AND GERMICIDES

Owing to the fact that the growth of *Mycoderma* on food storage brines causes deterioration and loss, it is desirable to ascertain whether growth can be prevented by the use of permissible antiseptics and in what manner the efficacy of such antiseptics is affected by the composition of the medium. For this reason the antiseptic power of certain chemicals toward the mixed *Mycodermas* normally occurring in salt stock brines was studied.

Comparison of Various Antiseptics.—The experiments with antiseptics were conducted first to compare the toxicity of a number of antiseptics to *Mycodermas* and secondly to determine the effect of salt concentration and pH value of the medium on the toxicity of sodium benzoate.

In the first experiment, salt stock brine procured from the California Conserving Company was used. This brine was 17° Baumé and showed a total acid content expressed as lactic of 0.46 grams per 100 cc.

The following antiseptics were added to this brine: acetic acid, citric acid, hydrochloric acid, lactic acid, oxalic acid, sulfurous acid, sodium sulfid, sodium thiosulfate, sodium sulfite, sodium metabisulfite, sodium cyanid, potassium permanganate, potassium chromate, mercuric chlorid, barium chlorid, calcium chlorid, cupric acetate, cupric sulfate, ferric sulfate, ferric chlorid, ferrous sulfate, potassium alum, zinc sulfate, formaldehyde, hydrogen peroxid, analine oil, carbon bisulfid, toluene, xylene, benzine, and phenol. Acetic and lactic acids were used in concentrations of .1–1.5 per cent hydrochloric, citric, and oxalic acids in concentrations of 0.1–1.0 per cent; the other acids and salts in concentrations of 0.01–0.10 per cent and mercuric chlorid in concentrations of 0.001–0.100 per cent. The oils such as carbon bisulfide, xylene, and toluene, were added as drops, 1–10 drops being used per 100 grams of brine.

As the brine used in the first series was poor in bacterial food and high in salt content, it was diluted to 10° Baumé and to each 1,000 cc was added 10 grams of dextrose, 1 gram peptone and 0.5 gram dipotassium phosphate. The resulting brine was 11° Baumé and contained 0.24 grams acid per 100 cc expressed as lactic. It was much more favorable to the growth of the *Mycoderma* present.

TABLE 14

CONCENTRATION OF VARIOUS ANTISEPTICS REQUIRED TO PREVENT GROWTH OF MIXED CULTURE OF MYCODERMA ON 17° BAUMÉ BRINE AND ON 11° BAUMÉ FORTIFIED BRINE

Antiseptic	Concentration necessary to prevent growth	
	On 17° Baumé per cent	On 11° Baumé per cent
Acetic acid.....	0.40	1.0
Citric acid.....	0.70
Hydrochloric acid.....	0.20	0.20
Hydrofluoric acid.....	0.06
Lactic acid.....	1.50
Oxalic acid.....	0.30	0.80
Sulfurous acid.....	0.01	0.06
Sodium benzoate.....	0.01
Sodium sulfite.....	0.01
Sodium metabisulfite.....	0.01
Mercuric chlorid.....	0.002	0.001
Formaldehyde.....	0.02	0.04
Hydrogen peroxid.....	0.06
Aniline oil.....	6 drops	6 drops
Benzine.....	30 drops
Toluene.....	8 drops	8 drops
Xylene.....	8 drops	6 drops
Phenol.....		0.06

The treated brines and untreated check samples were stored for a period of four months at room temperature. At the end of that period luxuriant growth had occurred in the untreated samples of brine. Growth was inhibited by the antiseptics listed in table 14 when used in the concentrations shown. Other antiseptics tested did not prevent growth in the concentrations used. Heavy growth consisting of the usual white *Mycoderma* film occurred in most samples in which insufficient antiseptic was used. In some samples the surface was not completely overgrown, although a white ring formed on the sides of the container and was taken as evidence of growth.

The following additional experiments were made: Salt itself was used as an antiseptic in one experiment and it was found that growth occurred at 21° Baumé after 1½ months incubation and at 22° Baumé after 4 months; 23° Baumé prevented growth.

Although growth was prevented by 0.06 per cent hydrofluoric acid it was not prevented by 0.10 per cent ammonium fluorid, the maximum concentration used.

Owing to its observed strong toxic effect further tests were made with sulfur dioxid solutions. Various amounts were added to 50 gram portions of salt stock brine of 18.3° Baumé and 0.08 per cent acidity. Growth occurred in all concentrations at the end of two weeks except at 0.035 per cent. In this same brine the addition of as much as 0.08 per cent sodium benzoate did not prevent growth. The addition of 0.02 per cent hydrofluoric acid prevented all growth in this brine.

The use of alkali as an antiseptic to prevent the growth of normally occurring organisms in salt stock brine was roughly determined. Salt stock brine was brought to the following pH values by the addition of sodium hydroxid; pH 4.5, 5.5, 7, 8.2, 9.0 and 9.8. After incubation at room temperature for four months, growth occurred at all pH values except the most alkaline, namely, pH 9.8.

The results obtained, especially those with sulfurous acid and sodium benzoate, indicate that the concentration of the antiseptics required to prevent *Mycoderma* growth varies considerably with the medium.

Of the antiseptics investigated, sulfurous acid, hydrofluoric acid, and sodium benzoate were the best both from standpoint of efficiency and of their probable effect on the pickles.

Of these three, sulfurous acid was most efficient, 0.04 per cent preventing all growth. When the brine was sufficiently acid, only 0.01 per cent sodium benzoate was required to inhibit growth. This is also true for the sulfites and sulfurous acid. However, when the acidity was low, even 0.08 per cent sodium benzoate did not prevent growth. Hydrofluoric acid was the most efficient of the mineral acids with the exception of sulfurous acid. Although 0.06 per cent hydrofluoric acid was required in one experiment, growth in solution of higher concentrations than 0.01 per cent was restricted to very slight ring formation. In concentrations of 0.02 per cent it practically inhibited growth.

Effect of Salt Concentration on the Toxicity of Sodium Benzoate.—Preliminary experiments showed that in cucumber juice of pH 5.1 and in the presence of 15 per cent salt, less than 0.1 per cent of sodium benzoate was required to inhibit growth. In solutions of lower salt content much larger amounts were necessary.

These observations led to the following experiments with cucumber-juice media of various salt and benzoate concentrations and inoculated with the following cultures:

- A*—Mixed *Mycodermas* freshly isolated from dill brine.
- B*—Mixed *Mycodermas* and mold spores from olive brine.
- C*—Organism No. 6 preserved in a 10 per cent NaCl cucumber-juice brine at 32° F.
- D*—Organism No. 3 preserved as above.
- E*—Organism No. 8 preserved as above.
- F*—Organism No. 21 from cider culture.

TABLE 15

EFFECT OF SALT CONCENTRATION ON THE CONCENTRATION OF SODIUM BENZOATE REQUIRED TO PREVENT GROWTH

Salt	Culture tested					
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>
	Concentration of sodium benzoate to prevent growth					
per cent	per cent	per cent	per cent	per cent	per cent	per cent
0.....	0.40	0.20	0.20	0.10	0.20	0.20
5.....	0.15	0.15	0.05	0.10	0.10	0.10
10.....	0.05	0.05	0.05	0.05	0.05	0.05
15.....	0.05	0.05	0.05	0.05	0.05	0.05

Cucumber juice was brought to pH 5.10 and 15 per cent salt content and to the plain cucumber juice as well as that containing added salt the following percentages of sodium benzoate were added: 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 respectively. The prepared media were then tubed, sterilized, and inoculated with the above cultures, and incubated for 10 days. At the end of this period members of this series showing no growth were re-inoculated. Final observations were made after an incubation period of one month. The amount of sodium benzoate necessary to inhibit growth is shown in table 15. Growth of all the organisms studied occurred in the absence of sodium benzoate at all salt concentrations.

The mixed cultures *A* exhibited somewhat greater resistance than the others to benzoate at 0 per cent and 5 per cent salt but at 10 per cent and 15 per cent salt all were of about the same resistance. Much less benzoate was required to prevent growth at 10 and 15 per cent salt than at 0 and 5 per cent.

Effect of pH Value on the Toxicity of Sodium Benzoate to Mycodermas.—To tubes of apple juice adjusted to various pH values by the addition of citric acid or of sodium hydroxid and containing various concentrations of sodium benzoate, was added a mixed culture of *Mycoderma* from dill brine and salt-stock brine. The tubes were incubated for 6 months and the presence or absence of growth noted with the results shown in table 16.

TABLE 16

EFFECT OF pH VALUE ON THE TOXICITY OF SODIUM BENZOATE TO MYCODERMAS

pH value	Benzoate required to prevent growth grams in 100 cc
2.7	0.05
3.8	0.06
4.7	0.50
5.4	1.00
7.3	4.00
7.8	More than 1.0
10.0	0.70

It will be seen that the pH value of the medium exerted a strong effect on the concentration of sodium benzoate required to prevent growth and that maximum tolerance lay at some pH value between 7.3 and 10.0.

EFFECT OF LIGHT

Although no extensive investigations on this point were conducted, it was observed that the growth of the organisms investigated took place in both diffused light and in the dark, but not in direct sunlight. This was found to be true both for liquid and solid media. This condition was also found true in practice since growth of *Mycoderma* and mold does not occur on salt stock tanks exposed to the sun.

DEATH-TEMPERATURE STUDIES

Although the death temperatures and the factors influencing them have been extensively studied for various bacteria, references to the death temperatures of yeasts and molds in the literature are not so numerous, and the available literature on the death temperature of *Mycodermas* is very scanty. In 1905, Takahashi⁽²¹⁾ isolated several varieties of *Mycodermas* from sake, kaji, and sake mash and studied their properties. He determined killing temperatures for his cul-

tures to range from 54.4° C to 60° C. Later the same author⁽²²⁾ isolated another *Mycoderma* which was killed in a moment at 70° C, or on exposure to 64° C for 5 minutes.

Ayers and associates⁽²⁰⁾ determined the death point of a culture of *Mycoderma* isolated from cloudy olive brine by heating in olive infusions containing various percentages of salt. They state that "In a 4 per cent salt brine the organism was not destroyed by heating the brine to 130° F (54.4° C) and maintaining this temperature for five minutes. However, in an 8 per cent salt brine it was destroyed at this temperature when maintained for 5 minutes." They found that in a 14 per cent salt brine the death temperature was lowered to 125° F provided this temperature was maintained for 10 minutes. Growth occurred in the 4 and 10 per cent salt brine after the same treatment.

TABLE 17
DEATH POINT OF MYCODERMAS IN CUCUMBER JUICE

Organism	Death point
<i>A</i>	60° C for 10 min.
<i>B</i>	60° C for 30 min.
6	50° C for 30 min.
3	60° C for 10 min.
8	60° C for 30 min.
21	65° C for 30 min.

In our investigations the death temperatures of five cultures of *Mycodermas* and one culture of *Penicillium* were determined. The cultures were designated *A*, *B*, 6, 3, 8, and 21 to conform with previous designations. *A* was mixed culture of *Mycoderma* yeast from 1928 dill brine and *B* was a mixed culture of mold spores and *Mycoderma* yeast from 1928 olive brine. 5 cc of sterile cucumber juice in small thin glass test tubes (1 cm in diameter and 12 cm long) were inoculated with small loopfuls of the organisms and heated in water baths held at 50, 55, 60, 65, 70, and 75° C respectively for 5, 10, and 30 minutes. Upon removal from the heating bath the tubes were immediately cooled in cold water and incubated at room temperature and were examined periodically until no further growth occurred. The results of the final observation are shown in table 17.

The death-temperature tests were then extended to include the effect of amount of inoculum, pH value of the juice and its salt content. Cucumber juice of pH 5.05 was brought to pH 2.75 by the addition of lactic acid and to pH 7.5 by the addition of sodium hydroxid. It was also brought to 2, 4, 6, 8, 10 and 12 per cent salt content. Five cubic centimeter portions of the prepared medium were

placed in test tubes (these tubes were larger than those used previously, measuring 1.6 cm in diameter, 15 cm long and 1.5 mm thick) and sterilized. The media were then inoculated with 5 drops of suspension of each of the cultures studied. This inoculum produced a turbid solution. The tubes were then heated for 5 minutes in water baths held at 50, 55, 60, 65, 70, and 75° C, respectively, and incubated. Distinct pellicle formation was taken as evidence of growth. The results are shown in table 18.

TABLE 18
EFFECT OF SALT AND pH ON DEATH TEMPERATURE

pH	Salt	Death temperature with 5 minutes' exposure					
		Organism					
		A	B	3	6	8	21
per cent	per cent	per cent	°C	°C	°C	°C	°C
2.75	0	55	55	55	50	50	50
5.05	0	70	70	65	65	60	75
7.5	0	70	70	65	65	65	75
5.05	0	70	70	65	60	60	75
5.05	2	70	70	65	60	60	75
5.00	4	70	65	65	60	60	75
5.05	6	70	65	65	60	60	75
5.05	8	70	65	65	55	60	75
5.05	10	65	65	65	55	60	55
5.05	12	60	60	60	55	60	55

The death points of the organisms studied were higher than those reported by Takahashi⁽²¹⁾ and Ayers⁽²⁰⁾ for their organisms. At a low pH the death temperature was considerably lowered. Moderate concentrations of salt did not affect the death temperature but high concentration lowered it considerably for most of the organisms studied.

CLASSIFICATION OF THE STRAINS OF MYCODERMA STUDIED

The various strains of *Mycoderma* resembled each other fairly closely in macroscopical appearance but differed in their microscopical appearance and in their tolerance for salt and for acid. In their tolerance for salt, especially, the organisms studied have retained the characteristics exhibited in their original environment.

On the basis of their tolerance for salt, the sixteen strains of *Mycoderma* studied may be classified into the following three groups:

1. Growth inhibited in cucumber juice by the addition of 10 per cent salt. Organisms 11, 12, 13, and 16 fall in this group.
2. Growth inhibited in cucumber juice by the addition of 15 per cent salt. Organisms 1, 2, 3, 4, 5, 6, 8, 14, and 15 fall in this group.
3. Growth inhibited in cucumber juice by the addition of 20 per cent salt. Organisms 7, 9, and 10 fall in this group.

In group 1, organism 13 differs from the other three in its lower salt tolerance, both in cucumber juice and in dill brine. In fact it had the lowest salt tolerance of all the organisms studied. Organisms 11, 12, and 16 are very similar in their salt tolerance. Organism 13 exhibited a maximum salt tolerance in cucumber juice of pH 3.2 and lower salt tolerance at other pH values. In cucumber-juice medium, organisms 16 oxidized lactic acid more rapidly than 13. In dill brine, organisms 11 and 12 oxidized the acids present at about the same rate. The initial rate was rapid but decreased with decrease in concentration of acid. In the case of organism 16, however, the rate of oxidation of acid was slow at first but increased as the concentration of acid decreased. Organism 13 oxidized lactic acid least rapidly of all the organisms in this group. Organism 11 was more tolerant for acetic acid than Nos. 12, 13, and 16, but less tolerant for citric acid. Organism 11, 13, and 16 were less tolerant for malic acid, oxalic acid, and tartaric acid than No. 12.

On cucumber juice, the films formed by organisms 11 and 13 are thin and only lightly wrinkled, particularly that of No. 13. They both show a turbid medium, much fine sediment, and a considerable growth on the walls of the tube. Organism 12 forms a thick, wrinkled film under which numerous granules form and settle to the bottom as a loose, granular sediment. Organism 16 forms a smooth, thin film, with heavy compact sediment. Unlike the others it produces notable quantities of gas. In microscopical appearance No. 13 differed in the predominance of short sausage-shaped cells, No. 16 in the predominance of branched chains of spherical to sausage-shaped cells. Organisms 12 and 13 were very similar to each other in size, No. 11 was smaller than the others and No. 16 differed from Nos. 11, 12 and 13 in the predominance of large spherical cells.

In group 2, organisms 3, 6, 14, and 15 were less salt tolerant both in cucumber juice and in dill brine than the others in this group. In dill brine, No. 5 was less salt tolerant than Nos. 1, 2, 4, and 8; No. 4 was the most salt tolerant of all organisms in dill brine. Organisms 1, 6, and 14 showed maximum salt tolerance in cucumber juice at pH

5.1 Organisms 1, 4, 8, and 14 oxidized lactic acid in cucumber juice more rapidly than Nos. 2, 5, 6, and 15, and organism 3 oxidized lactic acid in cucumber juice very slowly. In dill brine, organism 3 oxidized the acid slowly, also, but the others rather rapidly. These organisms were somewhat tolerant to acetic acid, Nos. 1, 2, and 3 being more so than the others; Nos. 6, 14, and 15 were more tolerant for citric acid than the others and No. 4 least tolerant; Nos. 2 and 6 were more tolerant for malic acid than the others and No. 8 least tolerant; No. 6 was found to be most tolerant for oxalic acid and No. 3 least tolerant; No. 14 was most tolerant for tartaric acid and Nos. 1, 4, 5, and 8 least.

On cucumber juice Nos. 1, 2, 4, 5, 6, and 8 grew similarly, No. 3 differed in that the film was thinner and only lightly wrinkled, the sediment was finer and the medium was more turbid; the film for No. 14 was thicker and only slightly wrinkled, there was but little sediment and much gas; No. 15 had only a slight film growth but a much heavier sediment and evolved gas. In microscopical appearance, Nos. 1, 4, and 6 were sausage-shaped to filamentous with very common chain formation; Nos. 2 and 8 were spherical to long rod-shaped with predominating sausage-shaped cells; No. 5 short sausage-shaped; No. 14 was sausage-shaped and the cells occurred in branched chains; Nos. 3 and 15 were spherical to ellipsoidal in shape. On the average No. 14 was considerably larger than the others.

In group 3 all the organisms were much less tolerant to salt in dill brine than in cucumber juice. The organisms of groups 1 and 2 were about equally tolerant in each, or even slightly more tolerant in dill brine. Organism No. 10 exhibited its maximum salt tolerance at pH 5.1 (natural cucumber juice) and had a lower salt tolerance in media of less and greater pH. Organism No. 9 oxidized lactic acid in cucumber juice more rapidly than did Nos. 7 and 10. In dill brine their rate of oxidation of acid was similar. They showed similar tolerance for acetic acid, citric acid, malic acid, oxalic acid, and tartaric acid. In the presence of 10 per cent salt, organism No. 10 was more tolerant for citric acid than No. 7. On cucumber juice, Nos. 9 and 10 differed from No. 7 in forming a more wrinkled, thicker film and a more compact sediment. In other media, growth was similar for all three. In microscopical appearance No. 9 differed from Nos. 7 and 10 in the predominance of short sausage-shaped cells and the cells were larger than either No. 7 or No. 10.

On the basis of this classification, the sixteen strains may be divided into varieties as follows:

Group	Variety	Organisms	Chief criteria for differentiation from other members of same group
1	A	13	{ Microscopical appearance, rate of oxidation of lactic acid, tolerance for acid and salt.
1	B	11, 12, 16	
2	C	1, 4, 6	
2	D	2, 3, 8, 15	{ Microscopical appearance.
2	E	5, 14	
3	F	9	{ Rate of oxidizing lactic acid and microscopical appearance.
3	G	7, 19	

The division of varieties *C*, *D*, and *E* was probably the most arbitrary as it was based chiefly on microscopical appearance. It is believed that with this exception the groups of organisms show sufficient difference in their properties to be classified into varieties and not merely designated as strains of the same species.

SUMMARY AND CONCLUSIONS

Some of the morphological and physiological properties of 16 strains of *Mycodermas*, one of *Torula* and 4 of molds from food-storage brines have been studied and the results reported in this paper.

1. The *Mycodermas* resembled each other fairly closely in macroscopical appearance but marked differences were found in their microscopical appearance. The molds were typical in appearance for the genera represented, namely, *Penicillium* and *Mucor*; the strain of *Torula* was also typical.

2. It has been found that the organisms studied retained the characteristics exhibited in their original environment, even after prolonged growth in various media.

3. The various strains of *Mycodermas* differed in their tolerance for salt and for acid.

4. All of the organisms studied were capable of growth in nutrient sucrose, dextrose, maltose, lactose, mannite, glycerin, acetic acid, citric acid, lactic acid, malic acid, oxalic acid, and tartaric acid. Growth in these media, in most cases, was not accompanied by visible gaseous fermentation of the medium.

5. Of the acids studied, citric acid was found most suitable for the development of the molds and lactic acid for the development of the *Mycodermas*, especially of strains isolated from pickle brines. Oxalic acid was most toxic. Acetic acid was less toxic than was expected. Many of the *Mycodermas* were capable of growth even in moderately high concentrations of this acid.

6. The concentration of salt necessary to inhibit growth of the various organisms was found to depend upon the composition of the

medium especially its pH value upon the manner of inoculating, and upon the previous method of growing the respective cultures. In general, the lower the pH of the medium, the lower was the concentration of salt required to inhibit growth. Although a few of the organisms were capable of growing in the presence of 20 per cent or more of salt, most of them were inhibited by the presence of 15 per cent salt.

7. The action of the organisms on the nutrient medium was apparently limited principally to the surface, for it was found that their activity was markedly influenced by disturbing the film or changing the ratio of surface to volume.

8. The activity of the organisms was retarded by the presence of a layer of neutral mineral oil. A certain minimum thickness of layer was found necessary to completely inhibit growth; this was 6 mm, under the conditions of our test.

9. The organisms were strongly aerobic and their development and activity was easily checked by limiting their oxygen supply.

10. No nitrogen fixation occurred in dilute cucumber juice.

11. Exposure to direct sunlight inhibited growth of all of the organisms studied.

12. Of the antiseptics studied, sodium benzoate, sulfurous acid, and hydrofluoric acid were most efficient and most practicable for factory use. Prevention of the growth of *Mycoderma* by the organic acids used or by salt was not found feasible, as too large a concentration of each is required when used alone. However, in the presence of moderately large amounts of salt, lower concentrations of acid are required. The toxicity of the antiseptics studied was increased by increasing the salt and acid content. This was especially true of sodium benzoate.

13. The death temperature of some of the organisms was determined. It was found to be influenced by pH, salt concentration, and amount of inoculum used.

14. The sixteen strains of *Mycoderma*s studied were classified into three groups and into seven varieties.

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COMPARISONS OF DAYTIME AND NIGHTTIME SOIL AND AIR TEMPERATURES

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INTRODUCTION

The importance of minimum, optimum, and maximum temperatures as well as of average temperatures for plant and animal life has been stressed by many writers. The time and rate of germination of seeds is without question largely dependent on soil temperature conditions. With most cultivated plants growth does not begin until a temperature of 40° to 50° Fahrenheit is reached by the soil.

Mosier and Gustafson⁽⁶⁾ state that growth is most vigorous at from 80° to 90° F, and that the soil nitrifying bacteria are most active at temperatures between 60° and 85° F. Other investigators give higher or lower ranges, as King⁽⁴⁾ who in referring to Ebermayer's investigations states that growth takes place most vigorously after the soil has reached 68° to 70° F, and that the maximum activity of the nitrifying bacteria occurs after a soil temperature of 98° has been reached; but if the soil reaches a temperature of 113° F their activity is nearly stopped, it being as weak as at 54°.

The effect of temperature on plant diseases and insect damage has received considerable attention. Smith⁽⁹⁾ shows that the effect of the temperature on parasites or fungous diseases of insects may be different from that on the insects themselves. He refers to the oat aphid which multiplies at a temperature of 40° or above, while the common parasite of this and of many other aphids is not active at a

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temperature below 56°. At a temperature of about 70°, however, the parasite will multiply approximately ten times as rapidly as its host. Jones, Johnson and Dickson⁽²⁾ point out that the general curve of plant disease development rises rather gradually to its optimum, then makes a sharp drop as temperature rises above this. The optimum they found was about 82° F for most of the diseases they investigated.

That temperature has an important effect on certain physical processes in soils has long been recognized. Bouyoucos⁽¹⁾ demonstrated that the rate of percolation of water increases with rise of temperature to about 86° F, and then decreases with further rise in temperature. This was true with a sandy loam, silt loam, clay loam, clay and muck soils while with a sandy soil the rate of flow increases with a constantly rising temperature. In the former soils he explains the results on the basis of the swelling of the colloidal material. The rate of flow of air through soils he found decreased with rise in temperature. King⁽⁴⁾ shows that soil ventilation due to diurnal changes in soil temperature will range from 0 up to possibly 20 cubic inches per square foot.

The importance of daytime and nighttime atmospheric or soil changes has received little consideration. Kincer⁽³⁾ has pointed out that in sections where the summer rainfall is not abundant, greater benefit to vegetation results if the rains come largely during the nighttime. There is not as great an evaporation from the soil and the cultivated soil is not so likely to be crusted as when the rains are of the daytime type where the effect of the hot sunshine often produces harmful results. Photosynthesis or the assimilation of carbon dioxide and a greater rate of transpiration by plants is a normal activity during the periods of sunlight according to the general principles of plant physiology. Mason⁽⁵⁾ reports that from records of the leaf elongation of palms in darkness it appears that such effects are diametrically opposed to the daylight activities. He has shown that the "inhibiting of the date-palm leaf growth in intense sunlight of the desert regions is due chiefly to the action of rays of light of definite wave length, that photosynthesis is most active in longer wave lengths and thus growth is inhibited by light that has but little potency in photosynthesis and conversely carbon assimilation is favored by light that has but little ability in inhibiting growth."

There seems to be a justification when considering the changes that are regularly taking place in the soil and air temperatures to consider the daytime hours and nighttime hours separately and not simply a certain daily (24 hours) maximum or minimum temperature.

METHOD OF OBTAINING TEMPERATURE DATA

Air temperatures were obtained by means of a thermograph placed in a U. S. Weather Bureau shelter at a height of 4½ feet above the soil surface. Soil temperatures were automatically recorded every 15 minutes by means of a Leeds and Northrup temperature recorder with electrical resistance thermometers placed at several depths in an area that was kept free of growing vegetation. Details concerning the area at Davis, California, where the observations were made have been described in a previous publication.⁽⁸⁾ The daily period of 24 hours was divided into two periods, the daytime or daylight hours and nighttime or the period between sunset and sunrise.

Portions of two years have been selected for this discussion, namely: February 20 to October 1, 1925, and January 1 to June 21, 1927. All of the temperature data obtained during these two years has been shown elsewhere⁽⁸⁾ by hour intervals, together with information on the character of the sky, general wind direction and periods of rainfall. The data herein reported were obtained by using all of the 15-minute records for each thermometer. In the 1925 period the shortest day was on September 30 when sunrise occurred at 6:01 and sunset at 5:51. The longest days were between June 16 and June 22 inclusive, when sunrise occurred at 4:39 or 4:40 and sunset at 7:34 or 7:35. The number of daylight hours therefore ranged from approximately 11 hours to 15 hours. During the 1927 period the shortest day was on January 1 when sunrise occurred at 7:26 and sunset at 4:54. The longest days were from June 16 to the end of the period. The number of daylight hours ranged from slightly over 9 hours to approximately 15 hours. The maximum air temperature for the day usually occurred several hours before sunset and for the night just after sunset. The minimum day and night air temperatures in general occurred around sunrise. The lag in the occurrence of the soil maximum and minimum temperatures at various depths with respect to the time of occurrence of the air maximum and minimums is approximately as follows: 3-inch depth—2 hours; 6-inch—4 hours; 12-inch—8 hours; 24-inch—70 hours; and 36-inch—80 hours.

It is clear from the preceding that daily soil temperature changes occur to a depth of 12 inches. At the 6-inch and 12-inch depths due to the lag as shown previously, the maximum soil temperatures will occur near or after sunset and therefore the night temperatures for these depths should average higher than the day temperatures. During

the warmer periods of the year the maximum soil temperature at a depth of 6 inches may occur approximately two hours before sunset.⁽⁷⁾ At this depth, therefore, the day and night maximum temperatures should be, in general, in close agreement.

TEMPERATURES IN 1925

The maximum, minimum and average soil and air temperatures obtained during the daytime and nighttime for each day of the period of February 20 to September 30 inclusive, 1925, are shown in figures 1-7 inclusive.

In order to fully understand the seasonal changes, tables 1-6 inclusive have been prepared. In these tables the highest and lowest maximums, minimums, and averages for the daytime and nighttime as well as the date of their occurrence, the usual maximums, minimums and averages between certain dates and the greatest spread which is the largest range in temperature between the maximum and minimum on one day are all shown for the period of February 20 to September 30 inclusive, 1925. In these tables special emphasis has been placed on the number of times when the air or soil temperatures were below 40° as most investigators usually mention some temperature around 40° as being the point where biological processes in the soil become active and that with air temperatures around 40° plant growth commences.

The minimum air temperatures and soil temperatures at a depth of one-half inch were at times below 40° during this 1925 period, but at a depth of 3 inches the minimum soil temperatures were never lower than 42°. For the soil depths beyond 3 inches the minimums were as follows: 6-inch depth—44°, 12-inch depth—48°, 24-inch depth—50°, and 36-inch depth—50°.

The average night temperatures at the 6-inch soil depth were usually higher than the average day temperatures, while at the ½ and 3-inch depths the reverse was true. The average day and night temperatures at the 6-inch depth were usually within 2° of each other and the same was true for the 12-inch soil depth.

A study of the figures and tables will show that there is no daily rise and fall in temperatures at the 24- and 36-inch depths in the soil. At these depths the temperature changes are slow and do not vary from day to day, as a general rule, more than 2°. On account of these facts only one curve is used to show the temperature changes at these depths and it is designated as "daily averages."

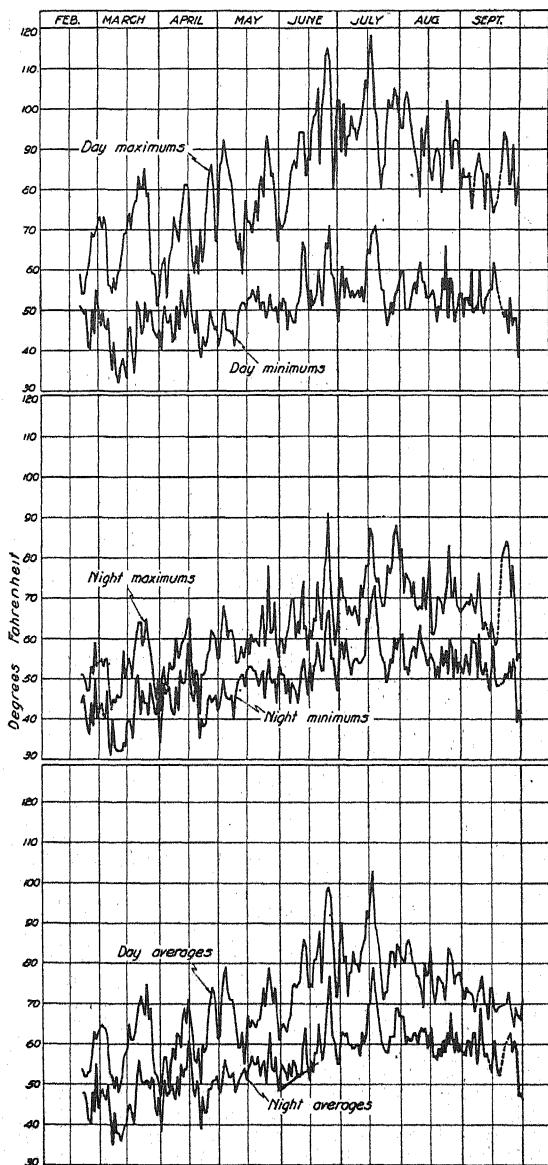


Fig. 1. Air temperatures for 1925 period.

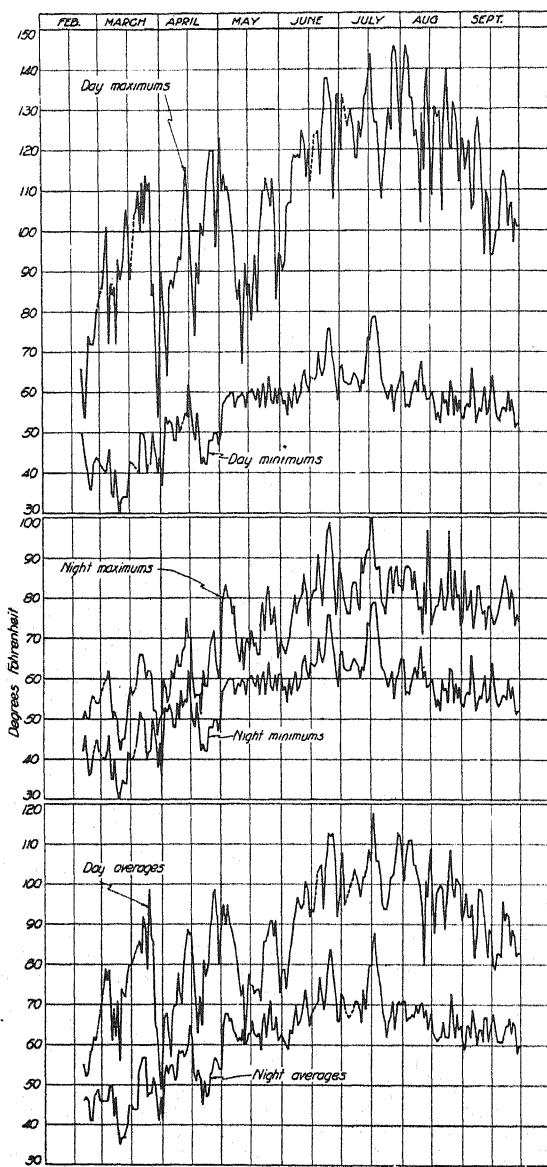


Fig. 2. Soil temperatures at $\frac{1}{2}$ -inch depth for 1925 period.

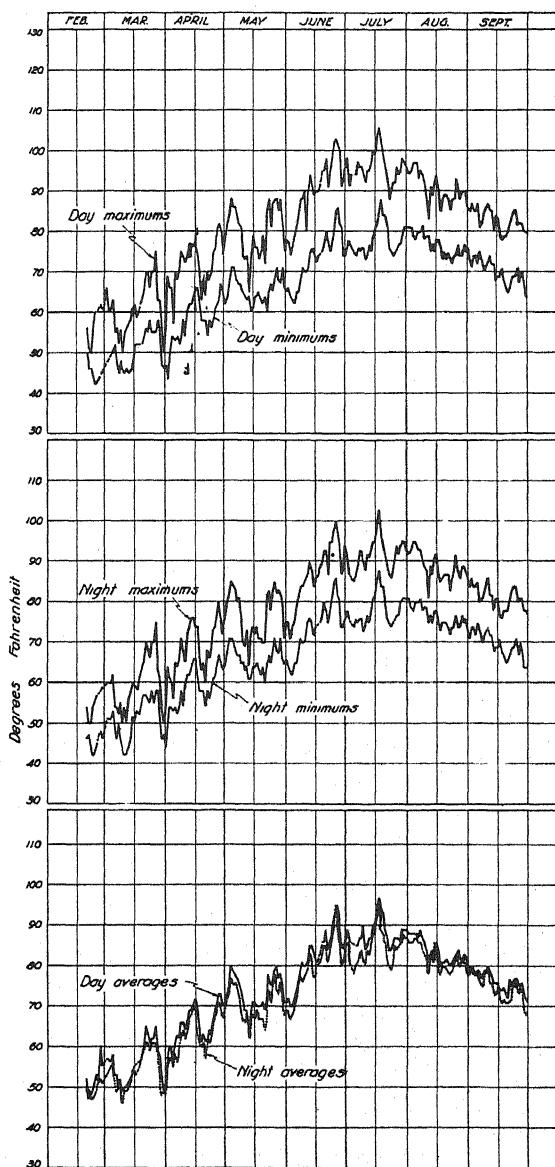


Fig. 3. Soil temperatures at 3-inch depth for 1925 period.

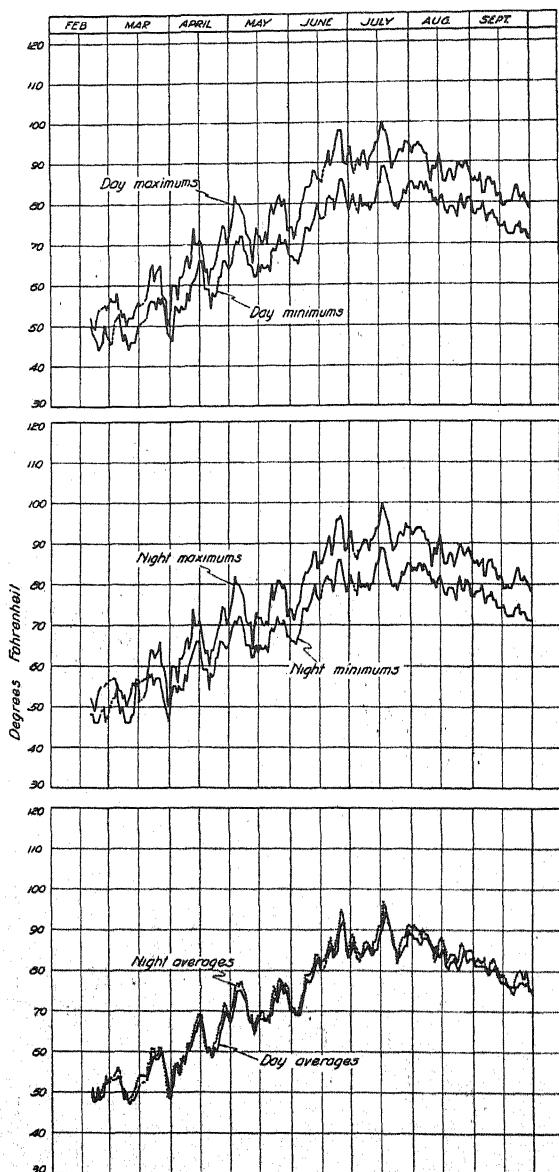


Fig. 4. Soil temperatures at 6-inch depth for 1925 period.

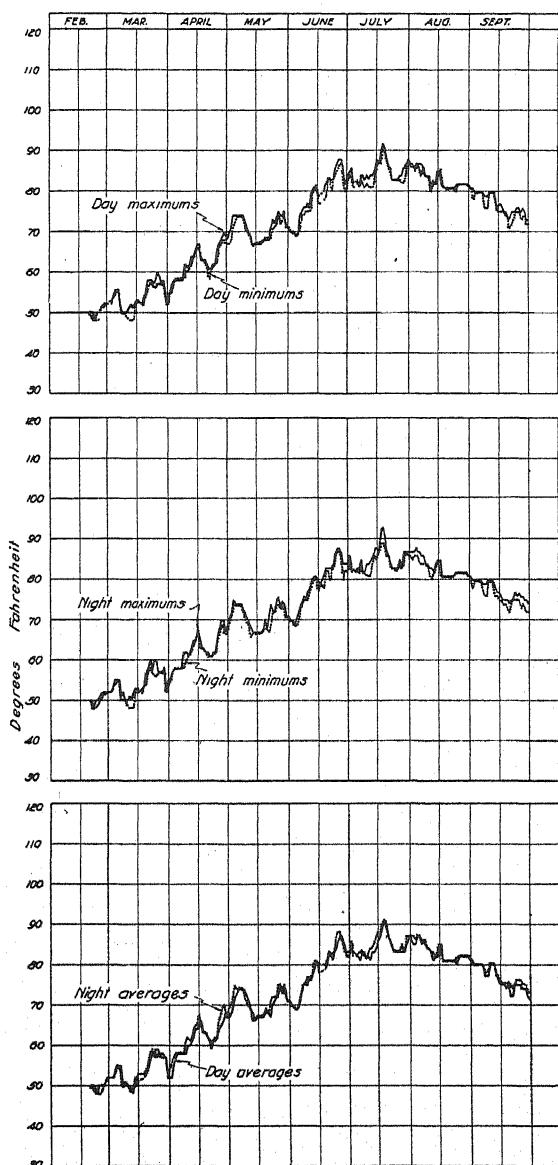


Fig. 5. Soil temperatures at 12-inch depth for 1925 period.

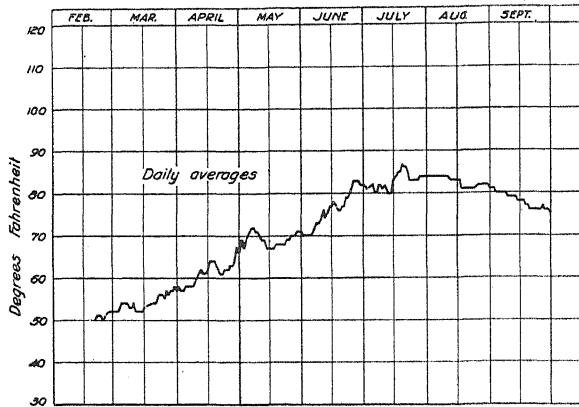


Fig. 6. Soil temperatures at 24-inch depth for 1925 period.

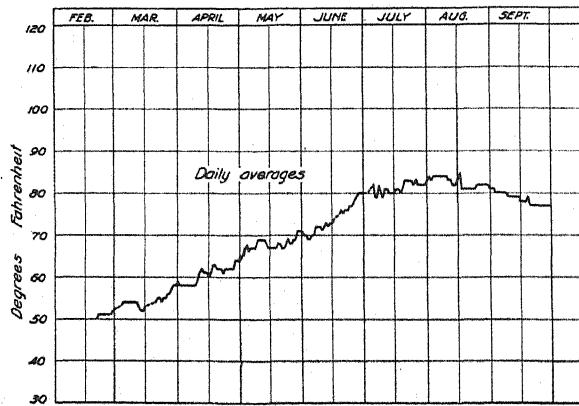


Fig. 7. Soil temperatures at 36-inch depth for 1925 period.

TABLE 1
DAYTIME AND NIGHTTIME AIR TEMPERATURES, FEBRUARY 20 TO SEPTEMBER 30, 1925

Daytime maximums						Daytime minimums						Greatest spread			
Highest	Lowest	Usual		Highest	Lowest	Usual		Tem.		Date	Tem.	Tem.		Date	Deg. Fahr.
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	March 7 to 19	April 21,	Less than 40°	July 26	54°	
July 17	117°	March 30	51°	February 20 to June 5	60°-80°	June 26, July 19	71°	March 10	32°	March 7 to 19	April 21,	Less than 40°	July 26	54°	
				June 5 to September 30	80°-100°					February 20 to June 9	February 20 to June 9	40°-50°			
										June 9 to September 30	June 9 to September 30	50°-60°			
Nighttime maximums						Nighttime minimums						Nighttime averages			
June 25	91°	April 20	41°	February 20 to June 1	50°-60°	July 18	73°	March 6	31°	March 5 to 17	March 31,	Less than 40°	July 29	31°	
				June 1 to September 30	60°-80°					April 20 to 23	September 28 to 30				
										February 20 to June 10	February 20 to June 10	40°-50°			
										June 10 to September 30	June 10 to September 30	50°-60°			
Daytime averages						Nighttime averages						Nighttime averages			
July 27	103°	April 1	47°	February 20 to June 5	50°-70°	July 17	79°	March 6	35°	March and April	Less than 40°	July 1	27°		
				June 5 to September 30	70°-90°					February 20 to May 1	February 20 to May 1	40°-60°			
										May 1 to July 1	May 1 to July 1	50°-60°			
										July 1 to September 1	July 1 to September 1	60°-70°			
										September 1 to 30	September 1 to 30	50°-60°			

TABLE 2

DAYTIME AND NIGHTTIME SOIL TEMPERATURES, $\frac{1}{2}$ INCH DEPTH, FEBRUARY 20 TO SEPTEMBER 30, 1925

Daytime maximums						Daytime minimums						Greatest spread	
Highest	Lowest		Usual		Highest	Lowest		Usual		Date	Date	Deg. Fahr.	
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Date	Deg. Fahr.	
July 28, August 5	146°	February 22, March 30	54°	February 20 to June 6	70°-110°	July 18, 19	79°	March 10	30°	11 times between February 24 and April 2	July 28	86°	
				June 6 to September 15	110°-140°					Less than 40°			
										February 20 to May 1	40°-50°		
										May 1 to September 30	50°-70°		
Nighttime maximums						Nighttime minimums						Nighttime averages	
July 17	100°	March 10	42°	February 20 to June 1	50°-70°	July 17, 18	79°	March 9	30°	12 times between February 23 and April 1	August 14	29°	
				June 1 to September 30	70°-90°					Less than 40°			
										February 20 to June 10	40°-60°		
										June 10 to August 10	60°-70°		
										August 10 to September 30	50°-60°		
Daytime averages						Nighttime averages						Nighttime averages	
July 17	118°	March 30	48°	February 20 to June 5	60°-90°	July 17	88°	March 9	35°	5 times around March 10	Less than 40°	March 24	51°
				June 5 to September 5	90°-110°					February 20 to May 1	40°-60°		
				September 5 to September 30	80°-90°					May 1 to September 30	60°-70°		

TABLE 3
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, 3-INCH DEPTH, FEBRUARY 20 TO SEPTEMBER 30, 1925

Daytime maximums						Daytime minimums						Greatest spread		
Highest	Lowest	Usual			Highest	Lowest	Usual			Date	Tem.	Date	Tem.	Deg. Fahr.
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Deg. Fahr.
July 17	107°	March 9, 30	50°	February 20 to June 5	50°-80°	July 18	88°	February 24	42°	Several times between February 20 and April 2	Less than 50°	June 24	21°	
				June 5 to September 30	80°-100°					February 20 to June 10	50°-70°			
								June 10 to September 30	70°-80°					
Nighttime maximums						Nighttime minimums						Nighttime averages		
July 17	103°	5 times between February 21 and March 30	50°	February 20 to June 5	50°-80°	July 17	88°	March 10	42°	Several times between February 20 and April 2	Less than 50°	March 26, June 7, 8	17°	
				June 5 to September 30	80°-95°					February 20 and June 10	50°-70°			
								June 10 to September 30	70°-80°					
Daytime averages						Nighttime averages								
July 17	97°	February 21	47°	February 20 to June 10	50°-80°	June 25, July 17	95°	March 14	46°	Several times between February 20 and April 1	Less than 50°	July 9	8°	
				June 10 to September 1	80°-90°					February 20 to June 10	50°-80°			
				September 1 to September 30	70°-80°					June 10 to September 1	80°-90°			
								September 1 to September 30	70°-80°					

TABLE 4
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, 6-INCH DEPTH, FEBRUARY 20 TO SEPTEMBER 30, 1925

Daytime maximums				Daytime minimums				Greatest spread			
Highest	Lowest	Usual		Highest	Lowest	Usual		Date	Tem.	Date	Deg. Fahr.
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	
July 17	101°	February 22	49°	February 20 to June 6	50°-80°	July 17, 18	89°	February 24, March 10	44°	February 20 to June 6	45°-70°
				June 6 to September 30	80°-95°			June 6 to September 30	70°-85°	June 23	15°
Nighttime maximums				Nighttime minimums				Nighttime averages			
July 17	100°	February 22	49°	February 20 to June 6	50°-80°	July 16, 17	89°	Several times between February 20 and March 21	46°	February 20 to June 6	50°-70°
				June 6 to September 30	80°-95°			June 6 to September 30	70°-85°	July 8	12°
Daytime averages				Nighttime averages				Nighttime averages			
July 17, 18	94°	March 10	47°	February 20 to June 6	50°-75°	July 17	97°	February 21, 22	48°	February 20 to June 6	50°-75°
				June 6 to September 5	80°-90°			June 6 to September 5	80°-90°	June 17	30°
				September 5 to September 30	70°-80°			September 5 to September 30	70°-80°		

TABLE 5
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, 12-INCH DEPTH, FEBRUARY 20 TO SEPTEMBER 30, 1925

Daytime maximums						Daytime minimums						Nighttime averages						Nighttime minimums			Nighttime averages		
Highest			Lowest			Usual			Highest			Lowest			Usual			Nighttime minimums			Nighttime averages		
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	February 28, March 12	February 28, March 12	February 28, March 12	September 15 to September 30	September 15 to September 30	September 15 to September 30
July 18	93°	February 28	48°	February 20 to June 6	50°-73°	July 17, 18	89°	February 22, March 11 to 13	48°	February 20 to June 6	50°-73°	July 18	91°	February 23	48°	February 20 to June 6	50°-75°	May 3	July 11	3°			
				June 6 to September 30	73°-88°					June 6 to September 30	73°-88°					June 6 to September 15	75°-91°						
																September 15 to September 30	70°-75°						

TABLE 6
DAILY AVERAGE SOIL TEMPERATURES, 24-INCH AND 36-INCH DEPTHS, FEBRUARY 20 SEPTEMBER 30, 1925

24-inch depth				36-inch depth			
Highest	Lowest	Usual		Highest	Lowest	Usual	
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.
July 19	87°	Several times between February 20 and March 14	50°-52°	February 20 to June 6	50°-70°	August 16	85°
						Several times between February 20 and March 14	50°-52°
						February 20 to June 6	50°-70°
						June 6,	75°-85°
						September 30	75°-82°
						June 6,	75°-82°
						September 30	75°-82°

DAYTIME AND NIGHTTIME SOIL AND AIR TEMPERATURES IN 1927

The maximum, minimum and average soil and air temperatures obtained during the daytime and nighttime for each day of the period of January 1 to June 21, 1927, are shown in figures 8-14 inclusive.

The seasonal changes are more clearly shown in tables 7-12 inclusive. In these the highest and lowest maximums, minimums and averages for the daytime and nighttime as well as the date of their occurrence, the usual maximums, minimums and averages between certain dates and the greatest spread which is the largest range in temperature between maximum and minimum on one day are all shown for the period of January 1 to June 21, 1927.

The minimum air temperatures and the soil temperatures at the $\frac{1}{2}$ -inch depth were below 40° several times during this 1927 period, but at a depth of 3 inches the minimum soil temperatures were below 40° only 3 times. For the soil depths beyond 3 inches the minimums, day or night were as follows: 6-inch depth— 41° ; 12-inch depth— 44° ; 24-inch depth— 48° ; and 36-inch depth— 52° .

The average day and night temperatures at the 6-inch depth were usually within 1° of each other, the night averages at this depth in general being higher than the day averages, while at the $\frac{1}{2}$ and 3-inch depths the reverse was usually the case. The average night temperatures at the 12-inch depth were usually 2° or less higher than the average day temperatures.

As previously stated a study of the figures will show that there is no daily rise and fall in temperature at the 24-inch and 36-inch depths and for this reason only one curve is used to show the temperature changes at these depths and it is designated as "daily averages."

DISCUSSION OF DATA

The occasional gaps in the data as shown by figures 1-14 are due to brief failures of the temperature recorder or thermograph. These are indicated on the graphs by an appropriate symbol (---). These missing data do not affect the conclusions which are drawn because there were relatively few times when they were of any duration.

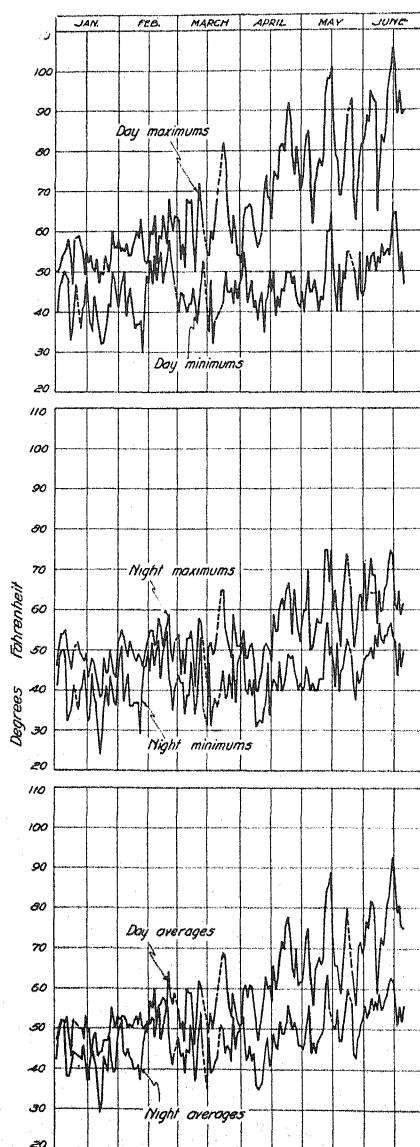


Fig. 8. Air temperatures for 1927 period.

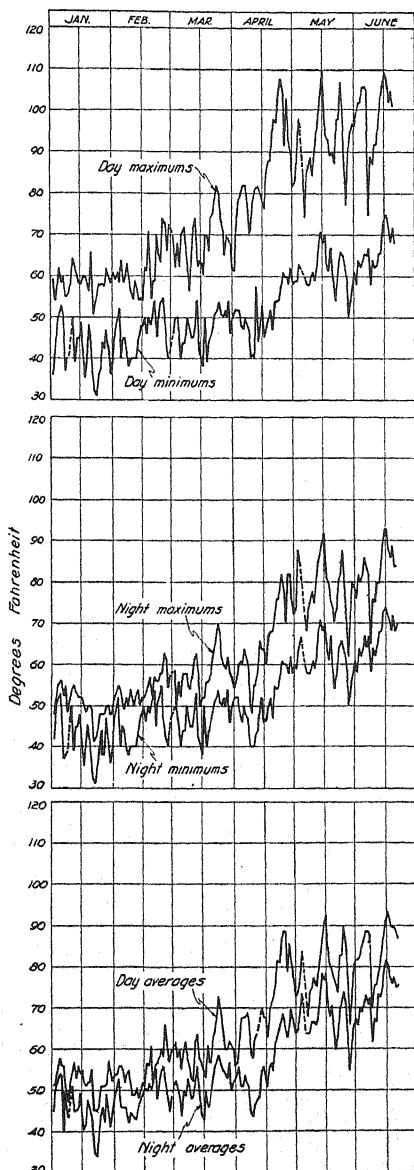


Fig. 9. Soil temperatures at $\frac{1}{2}$ -inch depth for 1927 period.

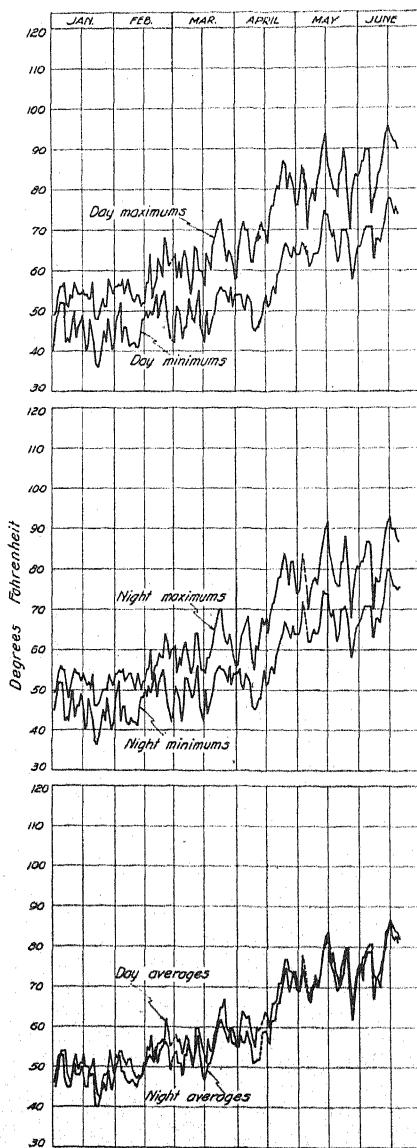


Fig. 10. Soil temperatures at 3-inch depth for 1927 period.

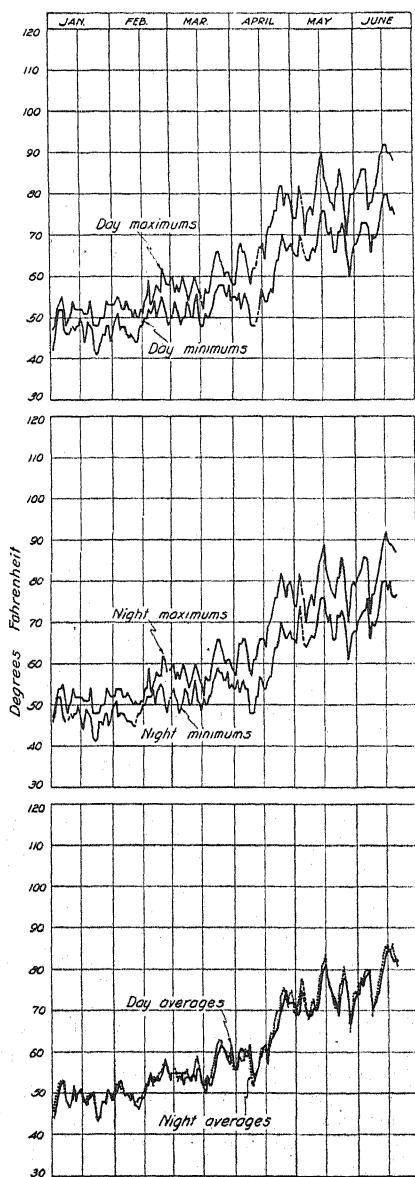


Fig. 11. Soil temperatures at 6-inch depth for 1927 period.

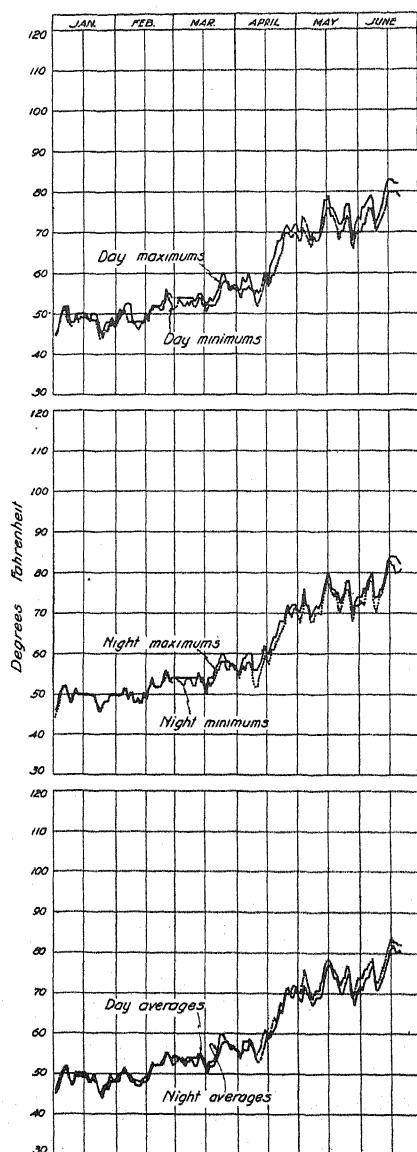


Fig. 12. Soil temperatures at 12-inch depth for 1927 period.

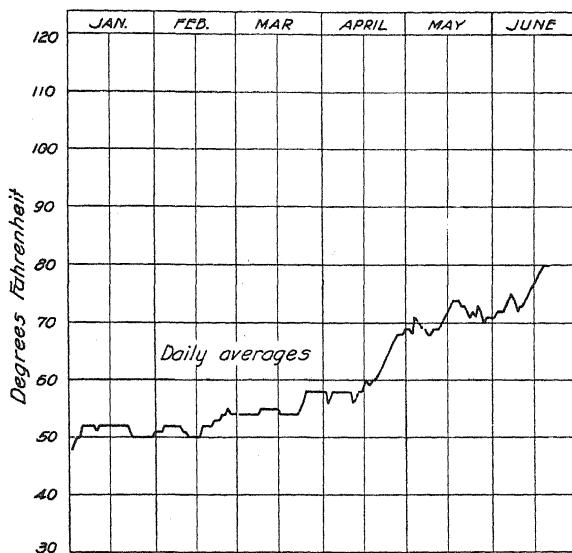


Fig. 13. Soil temperatures at 24-inch depth for 1927 period.

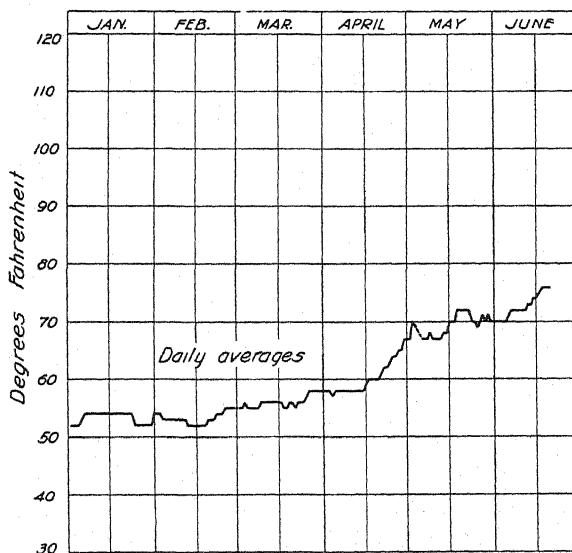


Fig. 14. Soil temperatures at 36-inch depth for 1927 period.

TABLE 7
DAYTIME AND NIGHTTIME AIR TEMPERATURES, JANUARY 1 TO JUNE 21, 1927

Daytime maximums						Daytime minimums						Greatest spread		
Highest	Lowest			Usual			Highest	Lowest			Usual			Date
Date	Tem.	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Deg. Fahr.
June 15	105°	January 8, 21	47°	January 1 to April 15	50°-70°	May 15, June 16, 17	65°	February 12	30°	25 times between January 7 and April 12	Less than 40°	April 14	42°	
				April 15 to June 21	70°-95°					January 1 to May 12	40°-50°			
										May 12 to June 21	45°-55°			
Nighttime maximums														Nighttime minimums
May 12, 13, 15 and June 14	75°	January 22	38°	January 1 to April 15	40°-55°	May 13, June 8, 14	57°	January 22	24°	48 times between January 6 and April 15	Less than 40°	May 4	26°	
				April 15 to June 21	55°-66°					January 1 to April 15	35°-50°			
										April 15 to June 21	40°-55°			
Daytime averages														Nighttime averages
June 15	93°	January 8, 18, 21	43°	January 1 to April 15	45°-60°	June 14	63°	January 22	29°	18 times between January 6 and April 11	Less than 40°	June 15	30°	
				April 15 to June 21	60°-80°					January 1 to April 15	38°-52°			
										April 15 to June 21	45°-60°			

TABLE 8
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, $\frac{1}{2}$ INCH DEPTH, JANUARY 1 TO JUNE 21, 1927

Daytime maximums						Daytime minimums						Greatest spread			
Highest	Lowest		Usual		Highest	Lowest		Usual		Date	Tem.	Date	Tem.	Date	Tem.
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	13 times between January 1 and March 18	Less than 40°	April 24	50°		
May 15, June 16	110°	January 21	50°	January 1 to April 15	55°-75°	June 15, 16	75°	January 23	32°						
				April 15 to June 21	80°-100°					January 1 to April 15	35°-55°				
										April 15 to June 21	55°-70°				
Nighttime maximums															
June 14, 15	93°	January 21	42°	January 1 to April 15	45°-65°	June 14	74°	January 22	32°	15 times between January 6 and March 15	Less than 40°	May 15	22°		
				April 15 to June 21	65°-90°					January 1 to April 15	40°-50°				
										April 15 to June 21	50°-70°				
Daytime averages															
June 16	94°	January 21, 22	45°	January 1 to April 15	50°-65°	June 14	82°	January 22	34°	January 21, 22, 23	Less than 40°	April 20	33°		
				April 15 to June 21	70°-90°					January 1 to April 15	40°-55°				
										April 15 to June 21	60°-80°				

TABLE 9
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, 3-INCH DEPTH, JANUARY 1 TO JUNE 21, 1927

Daytime maximums						Daytime minimums						Greatest spread	
Highest	Lowest		Usual		Highest	Lowest		Usual			Date	Deg. Fahr.	
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Date		
June 15	96°	January 22, 23	49°	January 1 to April 15	50°-70°	June 15, 16	78°	January 23	37°	January 22, 23, 24	Less than 40°	March 1	20°
				April 15 to June 21	70°-90°					January 1 to April 15	40°-55°		
										April 15 to June 21	55°-75°		
Nighttime maximums						Nighttime minimums							
June 15	93°	January 21, 22	46°	January 1 to April 15	50°-65°	June 14, 15	80°	January 22	37°	January 21, 22, 23	Less than 40°	May 15	18°
				April 15 to June 21	70°-90°					January 1 to April 15	40°-55°		
										April 15 to June 21	55°-75°		
Daytime averages						Nighttime averages							
June 15	87°	January 23	42°	January 1 to April 15	45°-63°	June 15	86°	January 21, 22	40°	January 1 to April 15	45°-60°	March 25, April 10	18°
				April 15 to June 21	67°-85°					April 15 to June 21	65°-83°		

TABLE 10
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, 6-INCH DEPTH, JANUARY 1 TO JUNE 21, 1927

Daytime maximums						Daytime minimums						Greatest spread		
Highest			Lowest			Highest			Lowest			Usual		
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Deg. Fahr.
June 15, 16	91°	January 1	47°	January 1 to April 16	50°-65°	June 15, 16	80°	January 23	41°	January 1 to April 16	45°-55°	May 15	14°	
				April 15 to June 21	70°-80°					April 15 to June 21	45°-55°			
Nighttime maximums						Nighttime minimums						Nighttime averages		
June 15	91°	January 1	47°	January 1 to April 16	50°-65°	June 13, 14, 15, 17	80°	January 22	41°	January 1 to April 16	45°-55°	May 15	13°	
				April 15 to June 21	70°-85°					April 15 to June 21	45°-55°			
June 15, 16	85°	January 23	43°	January 1 to April 15	45°-62°	June 15, 17	86°	January 23, 24	44°	January 1 to April 15	45°-52°	March 23, April 24	3°	
				April 15 to June 21	65°-85°					April 15 to June 21	65°-85°			

TABLE II
DAYTIME AND NIGHTTIME SOIL TEMPERATURES, 12-INCH DEPTH, JANUARY 1 TO JUNE 21, 1927

Daytime maximums						Daytime minimums						Greatest spread	
Highest	Lowest	Usual		Highest	Lowest	Usual							
Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Tem.	Date	Deg. Fahr.		
June 15, 16, 17	83°	January 1, 24	45°	January 1 to April 15	45°-60°	June 15 to 19	80°	January 23, 24	44°	January 1 to April 15	44°-60°	June 15, 16, 17	3°
				April 15 to June 21		65°-80°				April 15 to June 21	65°-80°		
Nighttime maximums						Nighttime minimums						Nighttime averages	
June 15, 18	84°	January 1, 23	46°	January 1 to April 15	46°-60°	June 15	83°	January 1	44°	January 1 to April 15	45°-60°	June 18	4°
				April 15 to June 21		65°-80°				April 15 to June 21	65°-80°		
Daytime averages						Nighttime averages						Nighttime averages	
June 16	82°	January 24	44°	January 1 to April 15	45°-60°	June 15	84°	January 23	45°	January 1 to April 15	45°-60°	March 23,	3°
				April 15 to June 21		65°-80°				April 15 to June 21	65°-80°	June 4	

TABLE 12
DAILY AVERAGE SOIL TEMPERATURES, 24-INCH AND 36-INCH DEPTHS, JANUARY 1 TO JUNE 21, 1927

24-inch depth				36-inch depth							
Highest	Lowest		Usual	Highest	Lowest		Usual				
Date	Date	Tem.	Date	Date	Tem.	Date	Tem.				
June 18, 20	80°	January 1	48°	January 1 to April 15	50°-60°	June 17 to 20	76°	January 1 to 4	52°	January 1 to April 15	52°-58°

The 1925 period under consideration extended from February 20 to September 30 inclusive. Where the ranges in temperature are given, as between two dates, it is to be understood that they indicate the general trend. By a close study of the figures one will find that there were a few days when these ranges or spreads were below or above those mentioned. The ranges during this 1925 period in the air day maximums were of about the same magnitude as those of the soil at the 3-inch depth, while the air day minimums were more comparable with those of the soil at the $\frac{1}{2}$ -inch depth. The night maximums and minimums for the air were of practically the same range as were the $\frac{1}{2}$ -inch soil temperatures. The average day air temperatures were of about the same magnitude as those of the soil at the 3-inch depth, while the average night air temperatures were more comparable with those of the soil at the $\frac{1}{2}$ -inch depth. At the 6-inch depth the average night temperatures were as much as 3° higher and at the 12-inch depth less than 2° higher than the average day temperatures. At the $\frac{1}{2}$ and 3-inch depths the average day temperatures were higher than the average night temperatures.

After about the first part of June, 1925, there was a distinct rise in the air and soil temperatures and after September 5 a noticeable drop.

The 1927 period extended from January 1 to June 20 inclusive and furnishes another period for comparisons of daytime and nighttime temperatures. This period, however, extends just up to the beginning of the warm summer and not through the summer as was the case with the 1925 period. The soil temperatures shown at the $\frac{1}{2}$ -inch depth are more comparable with the air temperatures than any of the other soil depths. This includes the maximums, minimums, and average temperatures during the daytime and nighttime. After about April 20, 1927, there was a distinct rise in the air and soil temperatures.

The daytime and nighttime maximums and minimums were lower in 1927, as a general rule, than in 1925. This is due to two factors: first, the 1925 period included more of the warmer months, and second, during the early part of the 1925 period there was different distribution of the rain and consequently different soil temperature conditions than in the corresponding portion of the 1927 period. In 1925 the number of days when it rained were few but on those days the rainfall was heavy, while in 1927 there were more days when it rained but the rainfall was light for any particular day. This has been previously shown, both by tables and figures.⁽⁸⁾

SUMMARY

This paper presents a comparison of air and soil temperatures at depths of $\frac{1}{2}$, 3, 6, 12, 24, and 36 inches in an area that was kept free of vegetation and not irrigated. Two periods were selected, one in 1925 from February 20 to September 30; and the second in 1927 from January 1 to June 20. The data presented are the maximum, minimum and average temperatures which were found between sunrise and sunset and which are called the daytime or day temperatures and from sunset to sunrise, the nighttime or night temperatures. It is the belief of the writer that a consideration of such temperature fluctuations is of importance as they may relate to plant and animal life.

In the 1925 period the number of daylight hours ranged from approximately 11 hours to 15 hours, while in the 1927 period which did not include as many of the warmer months they ranged from slightly over 9 hours to approximately 15 hours. The maximum air temperature for the daytime usually occurred several hours before sunset and for the night, just after sunset. The minimum day and night air temperatures in general occurred around sunrise. The maximum day soil temperatures at the $\frac{1}{2}$ - and 3-inch soil depths occurred before sunset while at the 6- and 12-inch depths due to the lag they usually occurred near or after sunset. The night temperatures for the 6- and 12-inch depths, therefore, averaged higher than the day temperatures.

In the 1925 period a day minimum air temperature of less than 40° occurred only a few times and at the $\frac{1}{2}$ -inch depth there were 11 times when it was below 40° . At depths of 3, 6, 12, 24, and 36 inches at no time was the day minimum below 42° . A night minimum air temperature of less than 40° occurred only a few times while at the $\frac{1}{2}$ -inch depth it occurred 12 times. At depths of 3, 6, 12, 24, and 36 inches the lowest night minimum was 42° for the 3-inch depth. The maximum day and night temperatures for the air and various soil depths varied considerably during the period.

The day average temperatures for the air, $\frac{1}{2}$ -inch, and 3-inch soil depths were higher than the night temperatures, while at the 6- and 12-inch depths the reverse was true in the 1925 period. At depths of 24 and 36 inches there was no regular rise and fall in the temperatures during a 24-hour period; simply either a gradual cooling or warming depending on the season of the year.

During the 1927 period a day minimum air temperature of less than 40° occurred 25 times, while at the ½-inch soil depth it occurred 13 times and at the 3-inch depth only three times. At depths of 6, 12, 24, and 36 inches at no time was the day minimum below 41°. A minimum night air temperature of less than 40° occurred 48 times, at the ½-inch depth 15 times, and only 3 times at the 3-inch depth. At depths of 6, 12, 24, and 36 inches the lowest night minimum was 41° at the 6-inch depth. The maximum day and night temperatures for the air and various soil depths were of less magnitude in the 1927 period than in the 1925 period.

The same fact was established by the 1927 data as was by the 1925 data relative to the relationship between the day and night average temperatures.

The general ranges in the day and night maximums, minimums and averages for the air and various soil depths are fully discussed for each of the two periods as well as the exact ranges for certain days when the largest or smallest ranges occurred.

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EFFECTS OF VARIOUS TREATMENTS ON THE CARBON DIOXIDE AND OXYGEN IN DORMANT POTATO TUBERS¹

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GENERAL PROBLEM AND LITERATURE

The marked influence of environmental conditions and treatments upon the length of the rest period of plants or plant parts is well known. Numerous investigations upon methods of abbreviating this rest period and hastening the initiation of growth have, however, failed to show convincingly the reason for the inception of this growth. Most of the previous investigations have been concerned with the changes in food reserves and enzyme activity. The present experiments deal primarily with the gaseous changes accompanying treatments which alter the rest period.

The literature of the subject frequently indicates that various outer tissues of seeds and tubers prolong the rest period by retarding the passage of carbon dioxide and oxygen. Several investigations with seeds (Crocker;⁽⁹⁾ Shull;^(26, 27, 28) Becker;⁽⁶⁾ Frietinger⁽¹³⁾) have shown that the permeability of the outer layer to gases plays an important rôle in the length of the rest period.

Studies with the potato tuber also suggest that the periderm and internal tissue may offer great resistance to the passage of carbon dioxide and oxygen and that the permeability may be changed by

¹ Paper presented to the Graduate School of the University of California in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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various treatments. Appleman⁽¹⁾ was able to shorten the rest period of potato tubers by various treatments such as removing the skin, cutting the tubers into pieces, subjecting them to subdued light, and wrapping them in cotton saturated with hydrogen peroxide. He suggests that these treatments facilitate the entrance of oxygen into the tuber or cause it to be liberated therein. He states further: "That a great increase in oxygen absorption actually occurs is proved conclusively by the effect of the various treatments on respiration, the rate of which was determined by the amount of carbon dioxide expired from the tubers Probably under normal conditions the skin becomes suberized before the completion of some growth mechanism requiring oxygen. The rate of oxygen diffusion through the suberized skin then determines the time required for the perfection of the growth mechanism." Rosa,⁽²⁴⁾ in a study of the relation of storage humidity to dormancy of potato tubers, suggests that besides inhibiting further suberization of the skin, the moisture may also make the skin more permeable.

Bartholomew,⁽⁵⁾ in a study of blackheart in potato tubers, ascribed the injury to a deficiency of oxygen caused by the increased rate of respiration of the tissues. The available supply of oxygen diffusing inward from the surface was used up before it reached the interior tissues. He also noticed that large tubers are more susceptible to injury than small ones. Stewart and Mix⁽²⁹⁾ also showed that deficient oxygen supply, and not accumulation of carbon dioxide, is responsible for the occurrence of blackheart. Bennett and Bartholomew⁽⁸⁾ found varietal differences in susceptibility to blackheart injury. They state that these differences in behavior probably result in part from differences in permeability of the skin of the tubers to oxygen.

Magness,⁽¹⁸⁾ working with apples and potato tubers, found that the percentage of carbon dioxide of the intercellular gas increases with rise in temperature; at 22° C there was 34 per cent in potato tubers. The percentage of oxygen decreases with increase in temperature and at 22° C was found to be 5.7. Removing the peel from the ends of apples greatly decreased the percentage of carbon dioxide and increased that of oxygen.

Davis⁽¹⁰⁾ found that the ratio of carbon dioxide to oxygen in the intercellular spaces of the potato varied with the conditions of storage, such as temperature and depth of piling, and with the age of the tubers. Following storage at 17°–18° C at the end of 16 hours at 45° C in atmosphere free from carbon dioxide and rich in oxygen,

the carbon dioxide content of the interior gas of the tubers had increased from 5 or 6 per cent to 50 per cent, and the oxygen percentage had decreased from 10 or 11 to about 4.

Stich,⁽³⁰⁾ working with potato tubers, and Johnstone,⁽¹⁵⁾ with sweet potato roots, showed that the increased rate of respiration on wounding results principally from mechanically facilitating the exchange of gases rather than from direct wound stimulation.

Wiesner and Molisch,⁽³¹⁾ using dry and moist pieces of periderm of potato tuber, found that carbon dioxide passed through the moist periderm several times faster than through the dry periderm. They conclude that gases pass through with increasing ease as additional amounts of water are absorbed by the cell wall. Loomis,⁽¹⁶⁾ in a study of the effect of humidity in storage upon the length of the rest period of potato tubers, states that at high temperatures the specimens stored in damp moss show less injury and make better growth than those stored dry. He further states that moist storage at these temperatures has no direct effect upon the rest period but probably results in more rapid formation of a wound periderm in the turgid tubers which prevents them from drying out. At moderate temperatures, however, the dry potatoes gave the best germination.

Several investigators, Shapovalov and Edson,⁽²⁵⁾ Priestley and Woffenden,⁽²²⁾ Artschwager,^(3, 4) Eames and MacDaniels,⁽¹²⁾ Rhodes,⁽²³⁾ and Herklotz,⁽¹⁴⁾ have published upon the anatomy of the periderm of the potato and the conditions conducive to rapid cork formation. They generally agree that the periderm is very impermeable to water and gases and that wound periderm formation is hastened by high humidity and high temperature.

Studies on the permeability of plant membranes to water and to other liquids may also furnish evidence as to their permeability to gases, for usually the plant tissues are permeable to gases only when the latter are in solution. Palladin⁽²¹⁾ points out the difference between diffusion of gases in the dissolved and undissolved states. In the dissolved state, the condition assumed usually to occur in plants, the velocity of movement of gases is directly proportional to the coefficient of solubility of the gas in the solvent in the septum. Not all cells and cell walls, however, are impregnated with water. Livingston³ states that diffusion of dissolved gases is possible if the gas is soluble in the membrane. When the latter contains water, this kind of diffusion can occur, for the gas dissolves in water. When the membrane

³ Livingston, B. E. Footnote, in: Palladin, V. I. Plant physiology. 2 ed. p. 107. P. Blakiston's Son and Company, Philadelphia. 1923.

contains little or no water, but contains some such material as suberin, and the gas dissolves in this material as it does in water, the action is similar to that of a wet membrane.

Nord and Franke,⁽²⁰⁾ in a study of the mechanism of enzyme action, used tobacco leaves and found that ethylene increased the permeability of the cells.

STATEMENT OF SPECIFIC PROBLEM

Because but little is known concerning the changes in permeability to gases, in gaseous exchange, and in composition of the gases in the interior of the tubers at the time when rest is broken, the present work was undertaken as an attempt to ascertain how various treatments affect the rate of loss of carbon dioxide and absorption of oxygen, the composition of interior gas of the tubers, the permeability of the periderm to gases, and the length of the rest period.

The treatments included harvesting at various stages of maturity, subjection to ethylene chlorhydrin vapor for 24 hours, storage dry and storage moist, storage in air of high oxygen percentage, and peeling the tubers.

EXPERIMENTS ON TUBERS HARVESTED AT VARIOUS STAGES OF MATURITY

Potato tubers of the varieties White Rose and Irish Cobbler were used throughout this study. In the maturity series the tubers were of the spring crop of 1928, in the remaining experiments, tubers of the fall crop of 1928, harvested November 10. In some experiments the White Rose variety only was used. All tubers selected were as nearly free from scab and mechanical injury as it was possible to obtain. For each series of experiments the same number of tubers was selected, and they were of approximately the same size and weight.

Experimental Procedure.—Tubers of the Irish Cobbler variety were dug at five different stages during the growth of the plants: first on May 21, when the vines were dark green in color and the tubers very immature, and then, with increasing maturity, on May 31, June 11, and June 21, and on July 2, when the vines were dead and the tubers considered mature. The White Rose tubers were dug on the same dates as the Irish Cobbler, and also on July 12, when the final harvest of mature tubers was made. On each harvest date a

sample of each variety was placed in storage at 25° C. The rate of respiration of the tubers was determined at approximately two-week intervals until some time after the rest period was broken.

Rate of Respiration.—For determining the amounts of carbon dioxide given off and of oxygen absorbed by the tubers, the Orsat gas analysis apparatus was used. The closed system was followed throughout. This method was checked against the continuous flow system, ascarite being used for the absorbing agent; and only a negligible difference between the two methods appeared when the carbon dioxide in the jars in the closed system was not allowed to become higher than 4 per cent.

TABLE 1

RESPIRATION RATE OF IRISH COBBLER AND WHITE ROSE TUBERS DURING THE GROWING PERIOD AND IN STORAGE AT 25° C

Expressed as milligrams of carbon dioxide per kilogram per hour

Variety	Date dug	At time of harvest	Fourteen-day periods after harvest					
			First	Second	Third	Fourth	Fifth	Sixth
Irish Cobbler.....	May 21	31.0	9.4	4.9	4.1	5.2	4.8*	6.7*
	May 31	19.4	6.2	5.3	4.1	4.7	5.4*
	June 11	11.8	5.1	4.6	4.6	5.5*
	June 21	15.1	5.9	4.3	5.4*
	July 2	12.2	5.5	5.2*	6.0*
	May 21	37.9	12.3	8.2	6.2	6.2	4.5*	6.8*
White Rose.....	May 31	27.3	10.8	6.3	5.0	4.2*	4.9*
	June 11	22.0	8.1	5.7	4.1	5.4*
	June 21	18.9	8.1	5.8	6.4*
	July 2	13.0	7.2	5.1*	4.6*
	July 12	10.5	7.6	7.4	7.2*

* All tubers sprouted.

Glass jars of one gallon capacity were fitted with No. 14 shellacked rubber stoppers, into which were fitted one or two metal stopcocks. Wire screen platforms were placed in the bottom of each jar, and each treatment was run in duplicate. The tubers were selected for uniform size and shape, and each lot of five tubers weighed between 600 and 700 grams. At each determination a 200 cc sample of gas was drawn from each jar, and 100 cc of this was analyzed for carbon dioxide and oxygen. Carbon dioxide was absorbed by a 50 per cent solution of potassium hydroxide, and oxygen was absorbed by a similar solution, to each 100 cc of which had been added 8 grams of pyrogallic acid. All determinations were corrected for differences in temperature and pressure, and the data are presented in terms of milligrams per kilogram per hour.

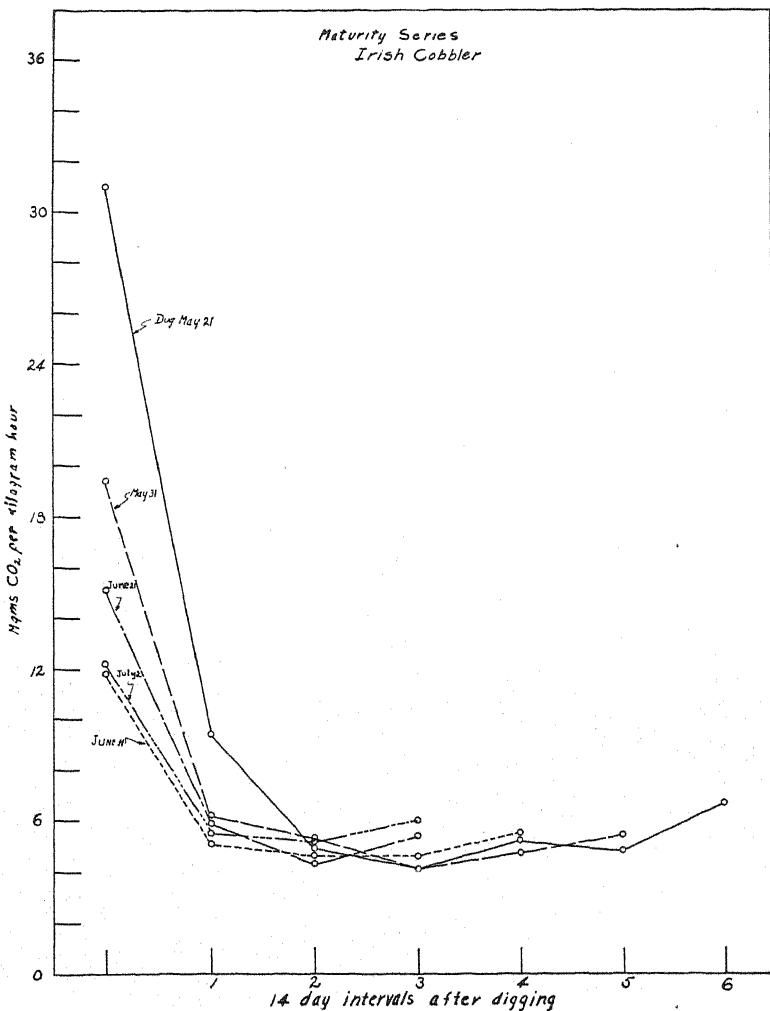


Fig. 1. Irish Cobbler tubers harvested at various stages of maturity. Respiration rates determined immediately after digging and during storage at 25° C.

Effects on Rate of Respiration.—The respiration data are presented in table 1 and shown graphically in figures 1 and 2. Respiratory activity is greatest in the earlier-harvested, less mature tubers, and gradually decreases as maturity advances. During storage also there is a gradual and regular decrease, until the respiration rates of tubers of all harvests are approximately equal at the end of the rest period. This decrease is extremely rapid immediately after harvest but diminishes during storage. The decrease in respiration rate of tubers in dry storage at 25° C is much more rapid than with those

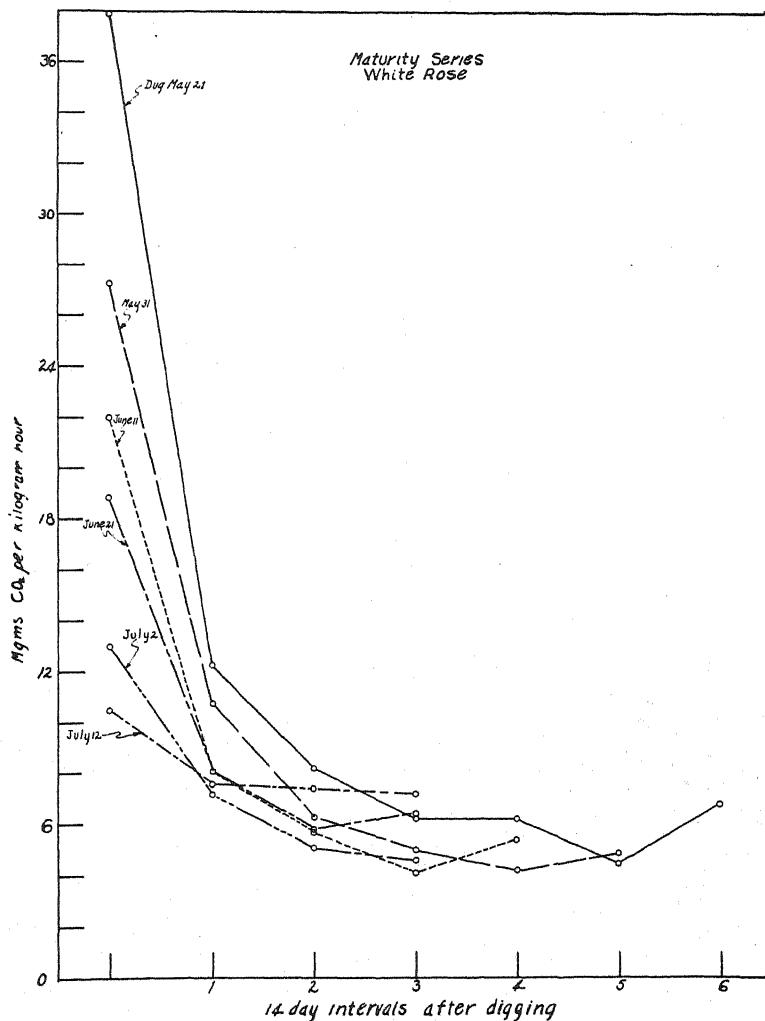


Fig. 2. White Rose tubers harvested at various stages of maturity. Respiration rates determined immediately after digging and during storage at 25° C.

that remain in the soil for the same length of time. In this respect, four to eight days in storage are equal to ten days in the soil. The rate usually increases slightly with resumption of growth. At every harvest period the White Rose tubers show greater respiratory activity than the Irish Cobbler; the former variety also has the shorter rest period. The data of table 1 also show that, with both varieties, the more mature the tuber is when harvested, the shorter is the rest period.

A COMPARISON OF WHITE ROSE AND IRISH COBBLER TUBERS UNTREATED WHEN STORED DRY AND STORED MOIST AND TREATED WITH ETHYLENE CHLORHYDRIN WHEN STORED DRY

Experimental Procedure.—Tubers of White Rose and Irish Cobbler varieties, dug November 10, were kept at 8° to 10° C until January 30, then treated and stored at 25° C to determine the differences in gaseous exchange of tubers and in composition of interior gases. The tubers were treated with 0.75 cc of 40 per cent ethylene chlorhydrin per liter of space for 24 hours, by the vapor method proposed by Denny.⁽¹¹⁾ Tubers of the dry-stored lots were stored in shallow wooden trays; those of the moist-stored lots, in wooden boxes in well-leached moist sphagnum moss or coarse, moist sawdust. The following lots of each variety were used; (1) chemically untreated, dry-stored; (2) chemically untreated, moist-stored; and (3) ethylene chlorhydrin-treated, dry-stored. The carbon dioxide given off, the oxygen absorbed, and the composition of interior gas were determined at intervals of three to seven days. The ethylene chlorhydrin vapor method, being the only chemical treatment given the tubers, will be designated throughout this paper as 'treated.' Dry storage and moist storage treatments will be referred to respectively as 'dry' and 'moist' and the chemically untreated tubers as 'untreated.'

Composition of Interior Gas.—Plugs of tissue from stem to eye end, one inch in diameter, were removed from five tubers of each lot for determination. One plug at a time was cut and pressed out of the brass borer under mercury into the gas extraction apparatus shown in figure 3. The operation of the apparatus is similar to that described by Magness.⁽¹⁸⁾ After two minutes' extraction in the Torricellian vacuum, the gas was collected in a gas burette. When samples of five tubers had been obtained, the gas was drawn into Henderson's respiratory gas analyzer (modified to have a capacity of 3.30 cc) and analyzed for carbon dioxide and oxygen. The absorbing agents for carbon dioxide and oxygen were the same as those used in the Orsat apparatus described above. These determinations were not made on the Irish Cobbler variety.

Effects on Rate of Respiration.—The Irish Cobbler untreated dry lots are, in general, higher in rate of loss of carbon dioxide and absorption of oxygen than similar lots of White Rose; with the untreated moist and the treated dry-stored lots, however, the reverse is true of

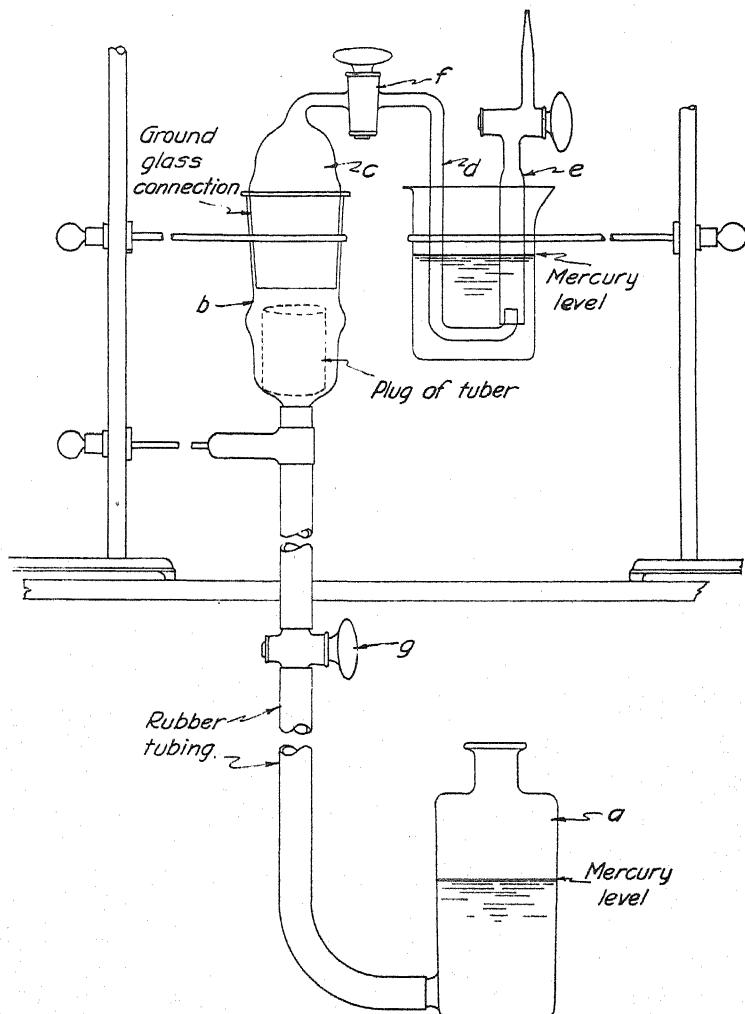


Fig. 3. Apparatus used for extracting gas from tubers. Cylinder (b) is partially filled with mercury by raising levelling bottle (a). The plug of tuber is then pushed from the cork borer under the mercury in cylinder (b), and stopper (c) is inserted and made tight. With stopcocks (f) and (g) open, the cylinder (c) and tube (d) are filled with mercury. Stopcock (f) is then closed and levelling bottle (a) is lowered, creating a Torricellian vacuum in tube (d) and part of cylinder (b). After two minutes, levelling bottle (a) is raised, stopcock (f) is opened, and the gas extracted from the tuber plug is collected and measured over mercury in burette (e).

the rates both of loss of carbon dioxide and absorption of oxygen. Data regarding length of rest period, composition of interior gas, and rates of gaseous exchange of the two varieties are shown in tables 2 to 5.

In general, the untreated dry lots gradually decrease during storage in rate of carbon dioxide given off and oxygen absorbed; the untreated moist lots increase slightly in rate of loss of carbon dioxide, but remain about the same or drop slightly in absorption of oxygen for a week or more, and then usually rise somewhat after the appear-

TABLE 2

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE
ROSE TUBERS TREATED JANUARY 30-31. STORED JANUARY 30

Treatment*	Jan. 31	Feb. 4	Feb. 7	Feb. 12	Feb. 19					
Milligrams carbon dioxide per kgm. hour respiration										
Untreated dry.....	8.1	6.2	4.8	5.9	5.4†					
Untreated moist.....		10.7	9.9	10.3†	10.5					
Treated dry.....		14.8	13.7	9.2†	8.3					
Milligrams oxygen per kgm. hour absorbed										
Untreated dry.....	8.4	6.2	6.7	5.5	5.2†					
Untreated moist.....		8.5	9.6	7.5†	8.9					
Treated dry.....		12.2	12.0	7.6†	7.6					
	Feb. 2	Feb. 4	Feb. 8	Feb. 13	Feb. 19					
Composition of interior gas, per cent										
CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	
Untreated dry.....	10.4	14.1	10.4	13.7	8.5	14.4	15.2	13.9	14.0	15.6
Untreated moist.....			22.4	11.7	15.0	12.0	17.9	12.2	18.8	10.2
Treated dry.....			13.3	12.5	10.8	14.5	15.5	13.6	10.3	16.6
Sum of percentages of carbon dioxide and oxygen in interior gas and CO ₂ /O ₂ ratio										
Ratio	Sum	Ratio	Sum	Ratio	Sum	Ratio	Sum	Ratio	Sum	
Untreated dry.....	0.74	24.5	0.76	24.1	0.59	22.9	1.10	29.1	0.90	29.6
Untreated moist.....			1.92	34.1	1.25	27.0	1.47	30.1	1.85	29.0
Treated dry.....			1.06	25.8	0.75	25.3	1.14	29.1	0.62	26.9

* These names are shortened designations for chemically untreated, dry-stored; chemically untreated, moist-stored; and ethylene-chlorhydrin-treated, dry-stored.

† All tubers sprouted. Sprouts removed on this date.

ance of sprouts. Treated dry lots increase rapidly within three or four days after treatment and then gradually decline in rates of loss of carbon dioxide and of absorption of oxygen. Several days more are required to show the full effects of moist storage upon rate of respiration than to show the effects of ethylene chlorhydrin treatment. With those treatments that abbreviate the rest period there is a striking increase in the rate of absorption of oxygen as well as an increase in the rate of loss of carbon dioxide.

Effects on Composition of Interior Gas.—The data of tables 2 and 3 show that the general tendency in all treatments of both varieties is for the carbon dioxide percentage of the interior gas to increase during storage, with the exception of a period just before or at the time

TABLE 3

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF IRISH COBBLER TUBERS TREATED JANUARY 30-31. STORED JANUARY 30

Treatment*	Jan. 31	Feb. 4	Feb. 7	Feb. 12	Feb. 19					
Milligrams carbon dioxide per kgm. hour respired										
Untreated dry.....	8.5	7.3	5.5	4.7	5.8†					
Untreated moist.....		7.3	10.1	8.7†	7.9					
Treated dry.....		12.4	9.0	8.6†	7.7					
Milligrams oxygen per kgm. hour absorbed										
Untreated dry.....	9.5	7.2	6.3	4.3	5.8†					
Untreated moist.....		6.7	8.9	6.3†	6.9					
Treated dry.....		10.9	9.4	7.0†	7.2					
	Feb. 2	Feb. 4	Feb. 7	Feb. 12	Feb. 19					
Composition of interior gas, per cent										
CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	
Untreated dry.....	12.1	15.6	15.0	13.2	8.0	14.7	13.1	14.5	16.7	13.1
Untreated moist.....			14.5	12.7	15.8	11.0	20.6	11.0	21.8	10.6
Treated dry.....			15.0	15.1	11.4	14.0	15.5	13.6	17.0	14.4
Sum of percentages of carbon dioxide and oxygen in interior gas and CO ₂ /O ₂ ratio										
Ratio	Sum	Ratio	Sum	Ratio	Sum	Ratio	Sum	Ratio	Sum	
Untreated dry.....	0.76	22.7	1.14	28.2	0.55	22.7	0.91	27.6	1.28	29.8
Untreated moist.....			1.14	27.2	1.44	26.8	1.87	31.6	2.06	32.4
Treated dry.....			0.99	30.1	0.81	25.4	1.14	29.1	1.17	31.6

* These names are shortened designations for chemically untreated, dry-stored; chemically untreated, moist-stored; and ethylene-chlorhydrin-treated, dry-stored.

† All tubers sprouted. Sprouts removed on this date.

the sprouts appear, when a decrease occurs, followed by an increase. No constant differences are shown between the two varieties in carbon dioxide or oxygen percentages.

In an attempt to find whether any of the treatments described above had affected the permeability of the tubers to carbon dioxide, every reading of carbon dioxide respiration and per cent of carbon dioxide in interior gas (see tables 2 and 3) of each of the treat-

TABLE 4

RELATIVE PERMEABILITY OF TREATED AND UNTREATED WHITE ROSE TUBERS TO CARBON DIOXIDE, FOUND BY COMPARISON OF EXPERIMENTALLY DETERMINED AND CALCULATED VALUES* OF PERCENTAGE OF CARBON DIOXIDE IN INTERIOR GAS OF TUBERS

Tubers treated and stored January 30-31

Treatments compared†	Per cent carbon dioxide in interior gas of the latter member of each comparison							
	Feb. 4		Feb. 7		Feb. 12		Feb. 19	
	Det.	Calc.	Det.	Calc.	Det.	Calc.	Det.	Calc.
Untreated dry vs. untreated moist.....	22.4	18.0	15.0	17.5	17.9	26.5	18.8	27.3
Untreated dry vs. treated dry.....	13.3	24.8	10.8	24.3	15.5	23.7	10.3	21.5
Untreated moist vs. treated dry.....	13.3	31.0	10.8	20.8	15.5	16.0	10.3	14.9

* Example.—Untreated dry compared with untreated moist expressed in the form of a proportion: (1) the rate of loss of carbon dioxide from untreated dry stored is to (2) the percentage of carbon dioxide in the interior gas of untreated dry stored as (3) the rate of loss of carbon dioxide from untreated moist stored is to (4) the percentage of carbon dioxide in the interior gas of untreated moist stored lots. By analyses these are found to be (1) (2) (3) (4) $6.2 : 10.4 : : 10.7 : 22.4$. This is not a true proportion, however, as the fourth term is too large. By calculation, the fourth term has a value of 18.0. If this calculated figure is larger than the figure determined by analysis, the tubers of the second member of the compared treatments, in the table above, are more permeable than the first; if smaller than the determined figure, those of the second member are less permeable than the first.

† These names are shortened designations for chemically-untreated, dry-stored; chemically-untreated, moist-stored; and ethylene-chlorhydrin-treated, dry-stored.

TABLE 5

RELATIVE PERMEABILITY OF UNTREATED AND TREATED IRISH COBBLER TUBERS TO CARBON DIOXIDE, FOUND BY COMPARISON OF EXPERIMENTALLY DETERMINED AND CALCULATED VALUES* OF PERCENTAGE OF CARBON DIOXIDE IN INTERIOR GAS OF TUBERS

Tubers treated and stored January 30-31

Treatments‡ compared	Per cent carbon dioxide in interior gas of the latter member of each comparison							
	Feb. 4		Feb. 7		Feb. 12		Feb. 19	
	Det.	Calc.	Det.	Calc.	Det.	Calc.	Det.	Calc.
Untreated dry vs. untreated moist.....	14.5	15.0	15.8	14.7	20.6	24.2	21.8	22.8
Untreated dry vs. treated dry.....	15.0	25.5	11.4	18.1	15.5	24.0	17.0	22.2
Untreated moist vs. treated dry.....	15.0	24.6	11.4	14.1	15.5	20.4	17.0	21.2

* The calculated values were obtained by ratios of rate of carbon dioxide loss and percentage of carbon dioxide in the interior gas of the compared treatments. A greater calculated than determined value indicates a probable greater permeability of the second member of the comparison. See footnote to table 4.

† These names are shortened designations for chemically untreated, dry-stored; chemically untreated, moist-stored; and ethylene-chlorhydrin-treated, dry-stored.

ments was compared with the readings of all the others. These experimentally determined and calculated values are presented and compared in tables 4 and 5. In all lots the weights, number, size, and shape of the tubers, and therefore the surface areas, were approximately equal. A calculated value lower than the experimentally determined value apparently indicates that the tubers of the lot of the second member (see tables 4 and 5) of each pair of compared members are less permeable to the gas than the tubers of the lot of the first member with which they are compared. The data of table 4 on White Rose indicate that for the first few days after storage, the tubers of the untreated moist lots are less permeable to carbon dioxide than the untreated dry lots, but thereafter surpass the untreated dry lots and become increasingly permeable during the storage period. Just after treatment, the treated dry lots become much more permeable than either the untreated dry or untreated moist lots; but gradually, during storage, they become less so. The data of table 5 on Irish Cobbler follow the same trend as those on White Rose, although in general they show less activity than the White Rose. Data on loss of carbon dioxide and absorption of oxygen in table 3 show very small differences early in the storage period between untreated dry and untreated moist lots. The comparisons of these two sets of data in tables 4 and 5 show also a less striking increase in permeability to carbon dioxide of the untreated moist over the untreated dry Irish Cobbler tubers than in the case of the White Rose tubers.

EXPERIMENTS ON WHITE ROSE TUBERS, TREATED AND UNTREATED, DRY AND MOIST-STORED

Experimental Procedure.—White Rose tubers, dug on November 10, were held at 8° to 10° C until February 8, 9, 10, and 11, when they were treated with ethylene chlorhydrin, stored at 25° C, and divided into the following four lots: (1) untreated dry, (2) untreated moist, (3) treated dry, and (4) treated moist. At four to seven-day intervals the tubers were analyzed for rate of loss of carbon dioxide, absorption of oxygen, and composition of interior gas. These determinations were replicated six times, with a total of 30 tubers for each determination when finally calculated.

Effects on Rate of Respiration.—The rates of loss of carbon dioxide and absorption of oxygen throughout the storage period (table 6) are typical of all the lots which have been described above in the series comparing White Rose and Irish Cobbler tubers untreated and treated with ethylene chlorhydrin when dry and when moist-

stored. The initial rate of loss of carbon dioxide and absorption of oxygen of the untreated moist lots is usually higher than that of the untreated dry lots; these rates, however, gradually increase in the moist lots but decrease in the dry lots. The effects of moist storage

TABLE 6

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE
ROSE TUBERS STORED AT 25° C, AVERAGE OF 6 REPLICATES

Treatment*	Feb. 9-10	Feb. 13-14	Feb. 17-18	Feb. 22-23	Feb. 27-28	March 6-7						
Milligrams carbon dioxide per kgm. hour respired												
Untreated dry.....	7.2	7.3	5.4	4.8	5.0†	4.2						
Untreated moist.....		8.6	8.9	10.7†	14.2	14.1						
Treated dry.....		9.0	6.6	5.8†	6.0	5.7						
Treated moist.....		13.1	11.5	12.7†	14.4	12.2						
Milligrams oxygen per kgm. hour absorbed												
Untreated dry.....	7.5	7.6	8.4	5.1	4.1†	4.6						
Untreated moist.....		7.7	8.1	8.4†	10.2	11.2						
Treated dry.....		9.1	7.1	6.9†	5.5	5.6						
Treated moist.....		10.1	9.6	8.5†	10.4	9.5						
Composition of interior gas in per cent												
	CO ₂	O ₂										
Untreated dry.....	2.5	18.8	6.4	16.2	7.5	15.7	6.0	15.1	8.8	14.5	12.1	15.3
Untreated moist.....			13.1	11.9	14.8	11.1	13.1	11.7	17.6	11.5	24.3	10.9
Treated dry.....			11.3	12.8	11.7	14.1	12.1	13.3	13.0	14.7	16.8	13.7
Treated moist.....			12.6	13.9	13.0	14.0	12.0	13.6	17.3	11.3	25.5	12.1
Sum of percentage of carbon dioxide and oxygen in interior gas and CO ₂ /O ₂ ratio												
	Sum	Ratio										
Untreated dry.....	21.3	0.13	22.6	0.39	23.2	0.48	21.1	0.40	23.4	0.60	27.4	0.79
Untreated moist.....			25.0	1.10	25.9	1.34	24.8	1.12	29.1	1.53	35.2	2.33
Treated dry.....			24.1	0.89	25.8	0.83	25.4	0.91	28.6	0.95	30.5	1.23
Treated moist.....			26.5	0.91	27.0	0.93	25.6	0.88	27.8	1.53	37.6	2.11

* These names are shortened designations for chemically untreated, dry-stored; chemically untreated, moist-stored; ethylene-chlorhydrin-treated, dry-stored; and ethylene-chlorhydrin-treated, moist-stored.

† All tubers sprouted. Sprouts removed on this date.

upon the rate of loss of carbon dioxide and absorption of oxygen are much more delayed than in the treatment with ethylene chlorhydrin. In all treatments that shorten the rest period more oxygen is absorbed than in the untreated dry lots.

Effects on Composition of Interior Gas.—With all treatments, the percentage of carbon dioxide gradually increases during storage, and then decreases about the time of sprout appearance, after which it

rises to a higher percentage than before the decline. Generally the percentage of carbon dioxide is higher in moist than in dry lots; higher in untreated moist than in treated dry lots; usually higher in untreated moist than treated moist lots in the early storage period, but somewhat lower than the latter after several weeks of storage. The percentage of oxygen is lowest as a rule in the untreated moist lots, although in general less carbon dioxide and more oxygen occur in the untreated moist than in the untreated dry lots in proportion to the amounts of carbon dioxide given off and oxygen absorbed by these lots.

TABLE 7

RELATIVE PERMEABILITY OF UNTREATED AND TREATED WHITE ROSE TUBERS TO CARBON DIOXIDE, FOUND BY COMPARISON OF EXPERIMENTALLY DETERMINED AND CALCULATED VALUES* OF PERCENTAGE OF CARBON DIOXIDE IN INTERIOR GAS OF TUBERS

Treated and stored at 25° C, February 8-11, averages of 6 replicates

Treatments† compared	Per cent carbon dioxide in interior gas of the latter member of each comparison									
	Feb. 13-14		Feb. 17-18		Feb. 22-23		Feb. 27-28		March 6-7	
	Det.	Calc.	Det.	Calc.	Det.	Calc.	Det.	Calc.	Det.	Calc.
Untreated dry vs. untreated moist.....	13.1	7.5	14.8	12.4	13.1	13.4	17.6	25.0	24.3	40.6
Untreated dry vs. treated dry.....	11.3	7.9	11.7	9.2	12.1	7.3	13.9	10.6	16.8	16.4
Untreated dry vs. treated moist.....	12.6	11.5	13.0	16.0	12.0	15.9	17.3	25.3	25.5	35.2
Untreated moist vs. treated dry.....	11.3	13.7	11.7	11.0	12.1	7.1	13.9	7.4	16.8	9.8
Untreated moist vs. treated moist.....	12.6	20.0	13.0	19.2	12.0	15.6	17.3	17.9	25.5	21.0
Treated dry vs. treated moist.....	12.6	16.5	13.0	20.4	12.0	26.5	17.3	33.4	25.5	36.0

* The calculated values were obtained by ratios of rate of carbon dioxide loss and percentage of carbon dioxide in the interior gas of the compared treatments. A greater calculated than determined value indicates a probable greater permeability of the second member of the comparison. See footnote to table 4.

† These names are shortened designations for chemically untreated, dry-stored; chemically untreated, moist-stored; ethylene-chlorhydrin-treated, dry-stored; and ethylene-chlorhydrin-treated, moist-treated.

In general the same relations exist between the ratios of the carbon dioxide to oxygen in the different lots as between the percentages of carbon dioxide in the interior gas of the tubers. The oxygen percentages of the tubers of the treated and moist lots do not decrease in so great amounts as the carbon dioxide increases in these same treatments. These relations are shown by the sums of carbon dioxide plus oxygen in the interior gas, which are usually of greater magnitude in the treated lots than in the untreated and also greater in the moist than in the dry lots.

In table 7 are presented the compared calculated and experimentally determined values which indicate the relative permeability

of the tubers in the various lots to carbon dioxide. These data indicate (1) that early in the storage period the untreated dry lots are more permeable than the untreated moist lots, but that after eight or nine days in storage the latter are more permeable; (2) that untreated dry lots are more permeable than treated dry lots, for the first few weeks of storage, but that after nearly four weeks the two are approximately equal; (3) that treated moist lots, with the exception of the first determination four days after treatment, are more permeable than the untreated dry lots and increase rapidly over the latter; (4) that untreated moist lots are more permeable than the treated dry lots, and become increasingly more so during storage; (5) that treated moist lots are more permeable than the untreated moist lots early after treatment and for several weeks thereafter, but gradually become less so until about four weeks after treatment, when the treated moist lots are less permeable than the untreated moist lots; and (6) that treated moist lots are consistently more permeable than the treated dry lots.

EXPERIMENTS ON DRY AND MOIST TUBERS IN JARS CONTAINING AIR AND A HIGH PERCENTAGE OF OXYGEN WITH AND WITHOUT THE ACCUMULATION OF CARBON DIOXIDE

Experimental Procedure.—White Rose tubers were dug on November 10. From November 10 to February 12 the tubers of the first test were stored at 8° to 10° C. On February 12 they were placed in storage at 25° C, and on February 20 they were divided into lots of four tubers each, weighing 677 grams per lot, which were sealed in one-gallon glass jars for a period of 48 hours: (1) air, KOH, dry; (2) air, no KOH, dry; (3) O₂, KOH, dry; (4) O₂, no KOH, dry; (5) O₂, KOH, moist; (6) O₂, no KOH, moist; (7) air, KOH, moist; (8) air, no KOH, moist. These abbreviations have the following meanings:

‘Air’: the gas surrounding the tubers was air, analyzing 20.6 per cent oxygen and 0.0 per cent carbon dioxide.

‘KOH’: 100 cc of a 50 per cent solution of potassium hydroxide was placed in the bottom of the jar.

‘Dry’: no moistening or drying agent was used.

‘No KOH’: no potassium hydroxide solution was used.

‘O₂’: the gas surrounding the tubers contained 60 per cent oxygen.

'Moist': the tubers were packed in moist sphagnum moss.

Another lot of tubers, not placed in jars, was used for analysis of interior gas as a check on those in the jars.

Four other experiments were run also, beginning February 14 and March 4, 6, and 8. In experiment 2, lots 6 and 8 were omitted.

Check jars without tubers but with moist moss were used to determine the amounts of carbon dioxide given off and of oxygen absorbed by the moss and water. The final data given in the tables are corrected for these figures. After 48 hours the amounts of carbon dioxide given off, the oxygen absorbed, and the composition of gas of the interior of the tubers were determined.

TABLE 8

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE ROSE TUBERS, FEBRUARY 14

Six tubers in each jar; weight 478-536 grams; stored January 24 at 25° C

Treatments*	Respiration rate		Composition of interior gas of tuber, per cent			Total CO ₂ plus O ₂	Composition of gas in jars when opened per cent		CO ₂ /O ₂ ratio of gas	
	Mgms. CO ₂ per kgm. hour	Mgms. O ₂ per kgm. hour	CO ₂	O ₂	N ₂		CO ₂	O ₂	From interior of tubers	In jar when op'n'd
Untreated (check).....			5.0	17.0	78.0	22.0	0.29
Air, KOH, dry.....		6.0	5.5	16.7	77.8	22.2	0.0	16.8	0.33
Air, no KOH, dry.....	4.9	5.3	13.6	18.9	72.5	27.5	2.2	17.8	0.98	0.12
O ₂ , KOH, dry.....		6.4	7.9	24.6	67.5	32.5	0.0	56.3	0.32
O ₂ , no KOH, dry.....	5.4	5.5	10.0	30.3	59.7	40.3	1.9	57.1	0.33	0.033
O ₂ , KOH, moist.....		29.7	17.6	17.9	64.5	35.5	0.0	36.8	0.98
Air, KOH, moist.....		25.8	6.1	8.2	85.7	14.3	0.0	0.3	0.75

* See text for explanation of treatments.

TABLE 9

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE ROSE TUBERS, FEBRUARY 20

Four tubers in each jar; weight 677 grams; stored February 12 at 25° C

Treatments*	Respiration rate		Composition of interior gas of tuber, per cent			Total CO ₂ plus O ₂	Composition of gas in jars when opened per cent		CO ₂ /O ₂ ratio of gas	
	Mgms. CO ₂ per kgm. hour	Mgms. O ₂ per kgm. hour	CO ₂	O ₂	N ₂		CO ₂	O ₂	From interior of tubers	In jar when op'n'd
Untreated (check).....			11.0	15.2	73.8	26.2	0.72
Air, KOH, dry.....		5.4	11.2	14.6	74.2	25.8	0.0	16.2	0.77
Air, no KOH, dry.....	4.8	5.3	20.3	13.0	66.7	33.3	2.8	16.4	1.56	0.17
O ₂ , KOH, dry.....		6.9	12.1	29.1	58.8	41.2	0.0	54.5	0.42
O ₂ , no KOH, dry.....	5.2	6.3	23.9	31.5	44.6	55.4	3.0	55.0	0.76	0.055
O ₂ , KOH, moist.....		13.9	27.4	22.1	50.5	49.5	0.0	42.4	1.24
O ₂ , no KOH, moist.....	5.3	10.9	27.6	24.2	48.2	51.8	4.2	51.1	1.14	0.082
Air, KOH, moist.....		12.2	17.9	10.6	71.5	28.5	0.0	5.0	1.69
Air, no KOH, moist.....	4.9	9.9	30.6	7.0	62.4	37.6	3.8	9.5	4.37	0.40

* See text for explanation of treatments.

TABLE 10

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE ROSE TUBERS; MARCH 4

Five tubers in each jar; weight 630 grams; stored February 22 at 25° C

Treatments*	Respiration rate		Composition of interior gas of tuber, per cent			Total CO ₂ plus O ₂	Composition of gas in jars when opened per cent		CO ₂ /O ₂ ratio of gas	
	Mgms. CO ₂ per kgm. hour	Mgms. O ₂ per kgm. hour	CO ₂	O ₂	N ₂		CO ₂	O ₂	From interior of tubers	In jar when op'n'd
Untreated (check).....	10.1	16.0	73.9	26.1	0.63
Air, KOH, dry.....	5.5	10.6	15.2	74.2	55.8	0.0	15.9	0.70
Air, no KOH, dry.....	4.9	19.4	13.7	66.9	33.1	2.4	16.2	1.42	0.150
O ₂ , KOH, dry.....	6.9	11.2	31.1	57.7	42.3	0.0	52.3	0.36
O ₂ , no KOH, dry.....	5.4	6.2	22.8	32.6	44.6	55.4	2.7	53.4	0.70	0.051
O ₂ , KOH, moist.....	14.8	26.1	24.2	49.7	50.3	0.0	41.2	1.08
O ₂ , no KOH, moist.....	5.5	12.2	27.0	26.8	46.2	53.8	3.8	50.3	1.01	0.076
Air, KOH, moist.....	13.6	16.0	11.8	72.2	27.8	0.0	5.1	1.36
Air, no KOH, moist.....	5.0	10.3	28.1	8.8	63.1	36.9	3.5	9.2	3.20	0.380

* See text for explanation of treatments.

TABLE 11

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE ROSE TUBERS; MARCH 6

Five tubers in each jar; weight 613 grams; stored March 3 at 25° C

Treatments*	Respiration rate		Composition of interior gas of tuber, per cent			Total CO ₂ plus O ₂	Composition of gas in jars when opened per cent		CO ₂ /O ₂ ratio of gas	
	Mgms. CO ₂ per kgm. hour	Mgms. O ₂ per kgm. hour	CO ₂	O ₂	N ₂		CO ₂	O ₂	From interior of tubers	In jar when op'n'd
Untreated (check).....	9.9	16.3	73.8	26.2	0.61
Air, KOH, dry.....	5.6	10.3	15.8	73.9	26.1	0.0	16.0	0.65
Air, no KOH, dry.....	5.0	5.4	18.8	13.6	67.6	32.4	2.3	16.3	1.39	0.14
O ₂ , KOH, dry.....	6.9	11.1	32.0	56.9	43.1	0.0	53.2	0.35
O ₂ , no KOH, dry.....	5.4	6.3	22.2	33.2	44.6	55.4	2.5	53.9	0.67	0.047
O ₂ , KOH, moist.....	15.1	24.5	25.3	50.2	49.8	0.0	42.1	0.97
O ₂ , no KOH, moist.....	5.8	12.6	26.0	27.1	46.9	53.1	3.7	50.9	0.96	0.073
Air, KOH, moist.....	14.1	16.1	12.3	71.7	28.3	0.0	6.2	1.31
Air, no KOH, moist.....	5.1	10.1	27.6	9.7	62.7	37.3	3.3	9.1	2.85	0.370

* See text for explanation of treatments.

Effects on Rate of Respiration.—The data presented in tables 8 to 12 indicate that the presence of potassium hydroxide solution in the respiration jars slightly increases the rate of oxygen absorption by the tubers. This is very strikingly shown by those lots surrounded by gas containing 60 per cent oxygen; they absorb more of this gas and give off slightly more carbon dioxide than those in air, which are otherwise receiving the same treatments. Moist tubers absorb oxygen and respire carbon dioxide more rapidly than the dry tubers.

Effect on Composition of Interior Gas.—The presence of potassium hydroxide in the jars lowers the percentage of carbon dioxide in the interior gas. The presence of the potassium hydroxide solution increases the percentage of oxygen found in the tubers surrounded by air; but the presence of the potassium hydroxide solution decreases the percentage of this gas found in the tubers surrounded by 60 per cent oxygen.

The percentage of oxygen in tubers surrounded by an atmosphere with a large proportion of this gas is higher, and the percentage of

TABLE 12

RELATION OF RESPIRATION RATES TO COMPOSITION OF INTERIOR GAS OF WHITE ROSE TUBERS; MARCH 8

Five tubers in each jar; weight 622 grams; stored March 3 at 25° C.

Treatments*	Respiration rate		Composition of interior gas of tuber, per cent			Total CO ₂ plus O ₂	Composition of gas in jars when opened per cent		CO ₂ /O ₂ ratio of gas	
	Mgms. CO ₂ per kgm. hour	Mgms. O ₂ per kgm. hour	CO ₂	O ₂	N ₂		CO ₂	O ₂	From interior of tubers	In jar when op'n'd
Untreated (check).....	10.2	16.2	73.6	26.4	0.63
Air, KOH, dry.....	5.7	10.5	15.5	74.0	26.0	0.0	16.2	0.68
Air, no KOH, dry.....	5.2	5.4	19.0	13.4	67.6	32.4	2.4	16.3	1.42	0.15
O ₂ , KOH, dry.....	6.8	11.6	31.8	56.6	43.4	0.0	51.7	0.37
O ₂ , no KOH, dry.....	5.6	6.2	22.4	33.3	44.3	55.7	2.6	52.3	0.67	0.05
O ₂ , KOH, moist.....	15.4	24.2	25.2	50.6	49.4	0.0	40.1	0.96
O ₂ , no KOH, moist.....	5.9	12.8	26.1	27.4	46.5	53.5	3.9	49.3	0.95	0.079
Air, KOH, moist.....	14.5	16.4	12.6	71.0	29.0	0.0	5.3	1.30
Air, no KOH, moist.....	5.2	10.2	27.7	9.9	62.4	37.6	3.6	9.5	2.80	0.38

* See text for explanation of treatments.

carbon dioxide, with the exception of one treatment, is also higher, than in tubers in air otherwise receiving the same treatment.

The percentage of carbon dioxide is higher and of oxygen lower in moist than in dry tubers; but the increase of rates of carbon dioxide loss and oxygen absorption is larger in the moist tubers than the increased percentage of carbon dioxide and decreased percentage of oxygen found in them, as compared with the dry tubers.

Only one treatment, 'air, KOH, moist,' had a higher percentage of oxygen in the tubers than in the surrounding atmosphere in the jar when the jar was opened. The small tubers with more surface exposed per unit of volume absorb oxygen at a higher rate than the large tubers.

PERMEABILITY OF PERIDERM TO GAS

Experimental Procedure.—Small, sound tubers, selected on the date of harvest, were stored at 8° to 10° C at intervals beginning November 10 with White Rose and December 1 with Irish Cobbler. These lots, in duplicate, of eight tubers each, approximately alike in size and shape, were selected for the same weight in each lot. Lot 1 was chemically untreated, stored dry; lot 2 was chemically untreated, stored moist; and lot 3 was treated with ethylene chlorhydrin and stored dry. All were stored at 25° C on the date of the first determination. At four to seven-day intervals, gas was extracted through the uninjured periderm for two minutes into a Torricellian vacuum with the instrument described and shown in figure 3. These gases were collected, and the amounts extracted from each lot were measured. Lots of White Rose were started on November 10, 22, and 27, on December 1 and 5, and on January 5, 9, 14, and 22. Lots of Irish Cobbler were started on December 1, 5, and 11, and on January 8. In order not to present so many figures, the tables give only the data on White Rose started on November 10, December 1, and January 14, and on Irish Cobbler on December 1 and January 8.

Small tubers similar to those used in the above experiments were studied in an effort to find what change, if any, in amounts and composition of gas extracted would result from the removal of a thin layer of periderm. Two lots of tubers of equal numbers, size, and total weight were selected, and the gas was drawn out, measured, and analyzed. One lot was then peeled, and the gases were immediately extracted again, measured, and analyzed. These lots were run in duplicate.

Effects on Amounts of Gas Extracted.—The data of these experiments are presented in tables 13 to 17. The amount of gas withdrawn from the White Rose tubers gradually diminishes with each lot as the interval between harvest and first extraction of gas increases, from November 10 to January 22. The same is true of Irish Cobbler tubers with gas withdrawn at intervals from December 1 to January 8. Because of careful selection of tubers in each lot for equality of size, shape, and weight, the differences in amount of gas withdrawn from the different lots at the first extraction before treatment were never more than 0.1 cc. Extractions made soon after harvest show that the amounts from the untreated dry lots slightly increased during storage at four or five-day intervals, but the lots started later after digging showed a steady decrease in the amount of gas extracted from the

untreated dry lots during the period of storage. Within each particular lot during the period of storage, and also in the initial extraction of each succeeding experiment, the untreated moist and treated dry lots give off more gas into the partial vacuum than the untreated

TABLE 13
AMOUNT OF GAS EXTRACTED FROM WHOLE WHITE ROSE TUBERS; TREATED AND STORED NOVEMBER 10

Eight tubers; 175 grams; average of duplicate samples

Treatment	Nov. 10	Nov. 15	Nov. 20	Nov. 24
	Cubic centimeters of gas extracted			
Chemically untreated, dry-stored.....	5.3	5.4	5.6	5.7
Chemically untreated, moist-stored.....	5.2	6.2	6.8	7.8
Ethylene-chlorhydrin-treated, dry-stored.....	5.2	6.2	6.7	7.3
	Per cent increase over chemically untreated, dry-stored			
Chemically untreated, moist-stored.....		14.8	21.4	36.9
Ethylene-chlorhydrin-treated, dry-stored.....		14.8	19.6	28.0

TABLE 14
AMOUNT OF GAS EXTRACTED FROM WHOLE WHITE ROSE TUBERS; TREATED AND STORED DECEMBER 1

Eight tubers; 180 grams; average of duplicate samples

Treatment	Dec. 1.	Dec. 5	Dec. 10	Dec. 15
	Cubic centimeters of gas extracted			
Chemically untreated, dry-stored.....	4.8	4.6	4.5	4.3
Chemically untreated, moist-stored.....	4.7	5.1	5.2	5.3
Ethylene-chlorhydrin-treated, dry-stored.....	4.8	5.0	5.0	5.0
	Per cent increase over chemically untreated, dry-stored			
Chemically untreated, moist-stored.....		10.8	15.5	23.2
Ethylene-chlorhydrin-treated, dry-stored.....		8.7	11.1	16.2

dry lots. The differences, however, become less as the end of the rest period is approached, until the gain of the treated and moist-stored lots over the untreated dry lots is negligible. During the early stages of the rest period, soon after harvest, more gas is extracted from the untreated moist-stored lots than from the treated, dry-stored; but later in the rest period, the treated dry-stored lots often give larger amounts of gas than the untreated moist-stored lots. Interestingly enough, those treatments that shorten the rest period

TABLE 15

AMOUNT OF GAS EXTRACTED FROM WHOLE WHITE ROSE TUBERS; TREATED AND STORED JANUARY 14

Eight tubers; 172 grams; average of duplicate samples

Treatment	Jan. 14	Jan. 19	Jan. 24	Jan. 28
Cubic centimeters of gas extracted				
Chemically untreated, dry-stored.....	4.5	4.1	3.9	3.6
Chemically untreated, moist-stored.....	4.4	4.1	4.2	3.9
Ethylene-chlorhydrin-treated, dry-stored.....	4.5	4.2	4.1	3.95
	Per cent increase over chemically untreated, dry-stored			
Chemically untreated, moist-stored.....		0.0	7.7	8.3
Ethylene-chlorhydrin-treated, dry-stored.....		2.4	5.1	9.7

TABLE 16

AMOUNT OF GAS EXTRACTED FROM WHOLE IRISH COBBLER TUBERS; TREATED AND STORED DECEMBER 1

Eight tubers; 180 grams; average of duplicate samples

Treatment	Dec. 1	Dec. 6	Dec. 11	Dec. 16
Cubic centimeters of gas extracted				
Chemically untreated, dry-stored.....	5.0	4.7	4.3	4.2
Chemically untreated, moist-stored.....	5.0	5.2	5.5	5.7
Ethylene-chlorhydrin-treated, dry-stored.....	4.9	5.3	5.5	5.6
	Per cent increase over chemically untreated, dry-stored			
Chemically untreated, moist-stored.....		10.6	27.9	35.7
Ethylene-chlorhydrin-treated, dry-stored.....		12.8	27.9	33.3

TABLE 17

AMOUNT OF GAS EXTRACTED FROM WHOLE IRISH COBBLER TUBERS; TREATED AND STORED JANUARY 8

Eight tubers; 170 grams; average of duplicate samples

Treatment	Jan. 8	Jan. 12	Jan. 17	Jan. 31
Cubic centimeters of gas extracted				
Chemically untreated, dry-stored.....	4.5	4.0	4.0	3.8
Chemically untreated, moist-stored.....	4.4	4.1	4.4	4.3
Ethylene-chlorhydrin-treated, dry-stored.....	4.4	4.4	4.5	4.3
	Per cent increase over chemically untreated, dry-stored			
Chemically untreated, moist-stored.....		2.5	10.0	13.1
Ethylene-chlorhydrin-treated, dry-stored.....		10.0	12.5	13.1

also increase the permeability of the skin to gas; and the time during the rest period when the treatments are made influences both the length of the rest period and the amount of gas extracted.

Data comparing the amounts and composition of gas withdrawn from peeled and unpeeled tubers are presented in tables 18 to 21. Removing the periderm and a thin layer of cortex increases the amount of gas extracted. As these increases are not of very great magnitude, the relative permeability of the tissue in the interior of the tubers is probably rather low and the periderm is most likely

TABLE 18

EFFECT OF PEELING ON AMOUNT AND COMPOSITION OF GAS EXTRACTED FROM
WHITE ROSE TUBERS, FEBRUARY 21

Twelve tubers in each lot; weight 265 grams; stored at 25° C; average
of duplicate samples

Treatment	Gas extracted, cc	Composition of interior gas, per cent		Total CO ₂ plus O ₂	Per cent N ₂ , by difference
		CO ₂	O ₂		
Lot 1	Before peeling.....	6.2	6.7	18.2	24.9
	Immediately after peeling.....	6.5	10.9	17.3	28.2
	Three days after peeling.....	5.75	8.8	18.7	26.9
Lot 2	Not peeled.....	5.95	5.8	19.1	24.9
	Not peeled (immediately after first extraction).....	5.85	8.9	19.4	23.3
	Not peeled, three days later.....	5.65	6.0	18.0	24.9

not the only portion of the tuber that offers resistance to the passage of gases. When gas is withdrawn from a given lot of tubers, with short intervening intervals, the amount extracted at each successive period is less than that from the preceding extraction; this fact suggests also that air cannot freely pass inward without some resistance. The amount of gas withdrawn again from tubers three or four days after peeling, is less than that withdrawn immediately after peeling, but somewhat more than that extracted from unpeeled tubers at the same time. Gas extracted from tubers immediately after peeling has a larger percentage of carbon dioxide than the gas withdrawn from the same lot just before peeling; on the other hand, when gas is extracted twice within a few minutes from the same lot of tubers without peeling or injuring, it has a lower percentage of carbon dioxide at the second extraction than at the first. With these treatments, the percentage of oxygen in the extracted gas does not change very much but usually decreases slightly in peeled tubers; with unpeeled tubers it increases somewhat in the extraction immediately after the first. Gases extracted and analyzed from peeled tubers three or four days after peeling have a higher carbon dioxide content and a somewhat higher oxygen content than those extracted from unpeeled tubers at the same time.

TABLE 19

EFFECT OF PEELING ON AMOUNT AND COMPOSITION OF GAS EXTRACTED FROM
WHITE ROSE TUBERS; FEBRUARY 25

Twelve tubers in each lot; weight 266 grams; stored at 25° C; average
of duplicate samples

Treatment	Gas extracted, cc	Composition of interior gas, per cent		Total CO ₂ plus O ₂	Per cent N ₂ , by difference
		CO ₂	O ₂		
Lot 3	Before peeling.....	6.15	11.5	22.4	77.6
	Immediately after peeling.....	6.75	23.0	33.6	66.4
	Four days after peeling.....	4.8	12.2	26.0	74.0
Lot 4	Not peeled.....	5.95	12.4	23.4	76.6
	Not peeled (immediately after first extraction)....	4.4	10.2	23.0	77.0
	Not peeled, four days later.....	4.5	11.3	23.9	76.1

TABLE 20

EFFECT OF PEELING ON AMOUNT AND COMPOSITION OF GAS EXTRACTED FROM
IRISH COBBLER TUBERS; FEBRUARY 26

Eight tubers in each lot; weight 249 grams; stored 8° to 10° C; average
of duplicate samples

Treatment	Gas extracted, cc	Composition of interior gas, per cent		Total CO ₂ plus O ₂	Per cent N ₂ , by difference
		CO ₂	O ₂		
Lot 5	Before peeling.....	6.45	1.8	23.0	77.0
	Immediately after peeling.....	6.45	4.2	23.9	76.1
Lot 6	Not peeled.....	6.25	3.5	22.7	77.3
	Not peeled (immediately after first extraction)....	6.20	1.8	23.0	77.0

TABLE 21

EFFECT OF PEELING ON AMOUNT AND COMPOSITION OF GAS EXTRACTED FROM
IRISH COBBLER TUBERS; FEBRUARY 26

Eight tubers in each lot; weight 240 grams; stored 8° to 10° C; average
of duplicate samples

Treatment	Gas extracted, cc	Composition of interior gas, per cent		Total CO ₂ plus O ₂	Per cent N ₂ , by difference
		CO ₂	O ₂		
Lot 7	Before peeling.....	6.25	8.5	29.0	77.5
	Before peeling (immediately after the first).....	6.20	1.9	22.8	77.2
	Immediately after peeling.....	6.5	4.9	25.2	74.8

DISCUSSION OF RESULTS

Some definite differences in rates of emission of carbon dioxide and absorption of oxygen, composition of interior gas, and permeability of tubers to gas have been found in tubers stored under various conditions and those treated with chemicals. These differences appear to be correlated with the length of the rest period.

Tubers treated with ethylene chlorhydrin and those chemically untreated are, when moist-stored, more permeable to the passage of gas than the chemically untreated dry-stored. Regardless of the method of treatment, the shorter the time between harvesting and the extraction of the gas, the more permeable are the tubers to the gas. The amount of gas extracted from the same lot of tubers also decreases throughout the storage period if the gas is first extracted and the potatoes stored late in the rest period. It was also found that White Rose and Irish Cobbler tubers have a higher respiratory activity when harvested immature, but that this decreases as maturity advances and also during subsequent storage at 25° C. These data suggest that the rate of respiration may be greatly influenced by the permeability of the tuber tissue to carbon dioxide and oxygen.

Appleman and Miller⁽²⁾ suggest that the higher respiration in immature Rural New Yorker potatoes for a period after digging may result from the fact that the skins are more permeable to gas. Removing the outer layer from small whole tubers with well developed periderm which was relatively low in permeability to gas, resulted in an increase in amounts of gas extracted similar to the increase in the case of the tubers which earlier during the rest period were chemically treated or stored moist. Three or four days after peeling, when the wound periderm had formed, the gas extracted from the peeled tubers decreased in quantity almost to the same level as that from unpeeled tubers. Analyses of these extracted gases indicate that the wound periderm that formed on the peeled tuber is similar to the periderm of the unpeeled tubers in its permeability to carbon dioxide and oxygen.

The increase in carbon dioxide immediately after peeling and the slight decrease in oxygen may be the result of increased respiration from wounding. This hypothesis does not appear plausible, however, because of the very short interval between peeling and the extraction of the gas. Stich⁽³⁰⁾ and Johnstone⁽¹⁵⁾ have shown that the increase

in rate of carbon dioxide given off after wounding largely results from mechanically facilitating the exchange of gases. The results of this investigation also suggest that the periderm of the tuber is more permeable to oxygen than to carbon dioxide, because the increase in percentage of carbon dioxide found is several times the decrease noted in oxygen. The data on unpeeled tubers with gas withdrawn again immediately after the first extraction suggest this also, for the carbon dioxide is lower and the oxygen percentage is higher in the second extraction than in the first.

This supposition is not borne out, however, by the studies on rates of diffusion of dissolved oxygen and carbon dioxide. Gases may, however, diffuse either in the dissolved or in the undissolved state. In the dissolved state, carbon dioxide would, because of its higher coefficient of solubility, diffuse at a greater rate than oxygen. The periderm of the potato tuber is, however, extremely dry, corky, and impervious to water. If the passage of gas through this tissue were in the undissolved state, oxygen would diffuse faster than carbon dioxide. The data of these experiments suggest that the passage of gas through the well-developed periderm of potato tubers may be at least partially in the undissolved state.

The work of Becquerel⁽⁷⁾ shows that thoroughly dried seed coats of certain plants are impervious to various gases, and Shull⁽²⁷⁾ states that no evidence was obtained of the diffusion of oxygen through absolutely dry seed coats of *Xanthium*. Similar data have been presented by Crocker,⁽⁹⁾ Shull,⁽²⁸⁾ and others working with seeds possessing unusually long rest periods. They found that the removal of the seed coats of *Xanthium* seeds induced oxygen entrance and germination to occur much sooner than when the seed coats were intact. Shull⁽²⁸⁾ also found that increased oxygen-pressure caused a large increase in the oxygen intake with the coats of *Xanthium* intact.

Investigation at this experiment station showed that potato tubers in gas of 60 per cent oxygen for a period of 48 hours absorbed more of this gas than those in air. This increase was not very great—about 1–2 milligrams per kilogram per hour. Perhaps a 48-hour exposure so late in the rest period is too brief to cause greater response. In other experiments in which tubers in the latter part of the rest period were kept in gas of 60 per cent oxygen, there was no apparent abbreviation of the rest period. Favorable results with high oxygen percentage might, however, be obtained if the tubers were exposed to the oxygen earlier in the rest period.

One should note that in all experiments of this investigation there is a larger oxygen absorption by the tubers subjected to those treat-

ments and conditions which shorten the rest period. This increased absorption of oxygen may be due, at least partially, to the increased permeability of the periderm to this gas. Conversely, the low permeability of the tissue to carbon dioxide probably plays a role in the metabolic processes of the tuber by the accumulation of this gas

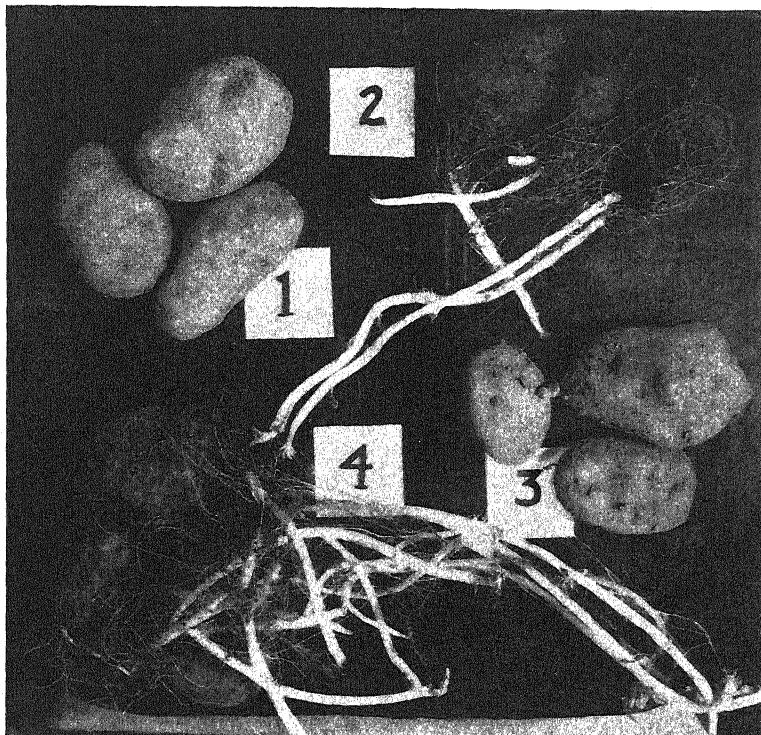


Fig. 4. White Rose potato tubers in 25° C storage, harvested November 10, treated November 28-29, stored November 29, 1928, and photographed January 15, 1929. (1) Chemically untreated, dry-stored; (2) chemically untreated, moist-stored; (3) ethylene-chlorhydrin-treated, dry-stored; (4) ethylene-chlorhydrin-treated, moist-stored. Note the dark color of the periderm and the prominent lenticels in the moist-stored tubers.

which may result in prolonging the rest period. Magness and Diehl⁽¹⁹⁾ are of the opinion that there may be a limitation in the oxygen supply which retards ripening of apples, or an increase in carbon dioxide which may inhibit or stop oxidation and further ripening. Willaman and Beaumont⁽²²⁾ also state that an accumulation of carbon dioxide in the atmosphere surrounding plant tissue appreciably affects the respiration of that tissue. Mack⁽¹⁷⁾ also has shown that after celery has been treated with ethylene, the removal of carbon dioxide by a solution of potassium hydroxide increases the rate of blanching.

Appleman⁽¹⁾ states that the treatments that shorten the rest period cause an increase in oxygen absorption; but he drew this conclusion only from the amount of carbon dioxide given off. Results of experiments with moist tubers, however, indicate that at least for short periods of time, the rate of carbon dioxide loss is not a reliable criterion for determining the amount of oxygen absorbed.

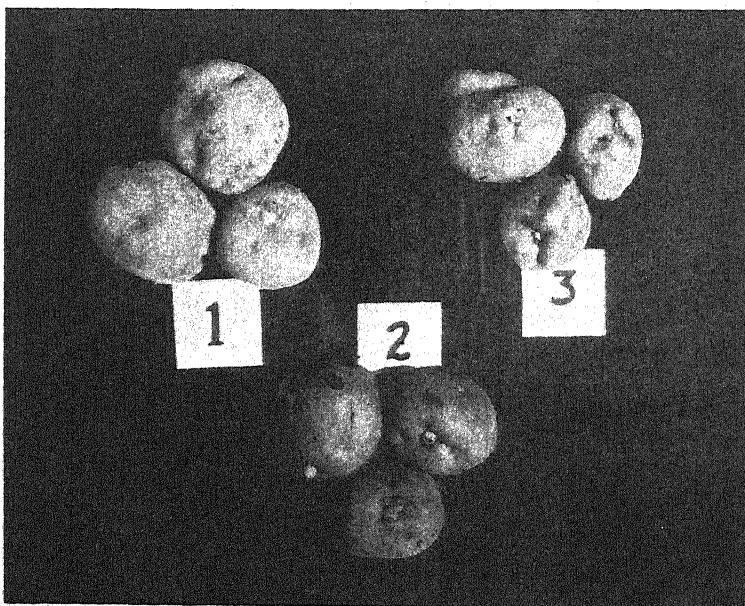


Fig. 5. Irish Cobbler potato tubers in 25° C storage, harvested November 10, treated December 10-11, stored December 11, 1928, and photographed January 15, 1929. (1) Chemically untreated, dry-stored; (2) chemically untreated, moist-stored; (3) ethylene-chlorhydrin-treated, dry-stored. Note the dark color and large, stubby sprouts of the untreated moist-stored tubers.

Moist storage of potato tubers as a means of abbreviating the rest period has not long been advocated. Rosa⁽²⁴⁾ states that moist storage, especially at moderate temperatures, has greatly shortened the rest period. Loomis,⁽¹⁶⁾ however, has concluded that at moderate temperatures the rest period was not shortened by storing potatoes in moist moss. In the investigations of the writer, however, moist storage at 25° C was one of the most effective methods of breaking the rest period. The behavior of moist-stored tubers, with regard to gaseous exchange and gaseous content, has been compared with that of tubers chemically treated and chemically untreated and stored dry. All lots of tubers stored moist have a greater rate of emission of carbon dioxide and absorption of oxygen than those stored dry. The

longer the time between harvest and moist storage, the longer it takes for the loss of carbon dioxide and absorption of oxygen to begin increasing and to reach the maximum rate.

When tubers were chemically treated and stored moist, the effects of both treatments were evident in rate of loss of carbon dioxide and absorption of oxygen. Keeping tubers in moist media facilitates the movement of carbon dioxide and oxygen through the periderm, apparently by increasing the permeability to these gases. These data suggest also that the increased rate of loss of carbon dioxide is a direct result of the increased absorption of oxygen. The data of the experiments in which the tubers were in the jar for a period of only 48 hours, show a much higher rate of absorption of oxygen by the moist than by the dry tubers, but only a small increase in rate of loss of carbon dioxide by the moist over those of dry storage. Analyses of the interior gas show, however, that within these 48 hours metabolic action has increased, as is manifested by the higher carbon dioxide and somewhat lower oxygen content in the moist than in the dry tubers. A longer exposure in the moist media would very likely result in a more rapid loss of carbon dioxide. In other experiments, tubers kept continuously moist for several weeks show a much increased rate of carbon dioxide loss parallel with the higher rate of absorption of oxygen.

The combined data of these two series of experiments indicate further that moist-stored tubers are at first more permeable to oxygen than the dry-stored; then follows a period when the differences decrease, and then a period when those stored moist continue to gain over those stored dry with both carbon dioxide and oxygen. Perhaps at the beginning of the experiments, when the moisture has not yet had sufficient time to penetrate the periderm, the two gases diffuse through only or mainly in the undissolved state. In this case, oxygen would pass through more rapidly than carbon dioxide. Later, however, when the moisture has penetrated the periderm, the gases will tend to diffuse through in the dissolved state. Carbon dioxide should then pass through the more rapidly. The amount of oxygen absorbed by the dry-stored lots decreases during storage, whereas that of the moist-stored lots increases. The partial pressure differences of the gases inside and outside of the tubers probably would account for some of these results, but data of those experiments in which a solution of potassium hydroxide was placed in the respiration jars, indicate that these differences do not result entirely from changes of partial pressure.

Tubers treated with ethylene-chlorhydrin and stored dry show behavior similar to that of the chemically untreated, moist-stored

tubers except that the former are usually more rapid in their action immediately after treatment and storage and that after the initial rise they gradually decline to a lower level than the action of the chemically untreated, moist-stored and ethylene-chlorhydrin-treated, moist-stored lots.

Tubers of the ethylene-chlorhydrin-treated, moist-stored lots had the shortest rest period. Next in order followed the chemically untreated, moist-stored, the ethylene-chlorhydrin-treated, dry-stored, and the chemically untreated, dry-stored, when all were stored at 25° C. Photographs of tubers treated in this manner are shown in figures 4 and 5.

SUMMARY AND CONCLUSIONS

White Rose and Irish Cobbler tubers were used in an attempt to ascertain how various treatments affect the rate of loss of carbon dioxide and absorption of oxygen, the composition of interior gas of the tubers, the permeability of the periderm to gases, and the length of the rest period. The treatments included harvesting at various stages of maturity, subjection to ethylene chlorhydrin vapor for 24 hours, storage dry and storage moist, storage in air of high oxygen percentage, and peeling the tubers.

Irish Cobbler tubers were dug at various stages of maturity on May 21 and 31, June 11 and 21, and July 2; the White Rose tubers were dug on the same dates as the Irish Cobbler and also on July 12.

Chemically treated tubers were subjected to 0.75 cc of 40 per cent ethylene chlorhydrin per liter of space for 24 hours.

Tubers of the dry-stored lots were stored in shallow wooden trays; those of the moist-stored lots, in wooden boxes in well-leached moist sphagnum moss or coarse, moist sawdust.

Dry and moist tubers were placed in respiration jars containing air with 20.6 per cent and 60.0 per cent oxygen with and without the accumulation of carbon dioxide.

The permeability of the periderm of the tubers to gas was determined by withdrawing the gas from the tubers into a vacuum from whole uninjured tubers and from peeled tubers.

The respiratory gases were analyzed for carbon dioxide and oxygen with the Orsat gas analysis apparatus. The gases extracted from the tubers into a vacuum were analyzed for carbon dioxide and oxygen with a modified Henderson's Respiratory Gas Analyzer.

White Rose and Irish Cobbler tubers harvested immature exhibit a higher respiratory activity at time of digging than those harvested more mature. The respiration rate gradually declines with advancing

maturity in the field and in storage at 25° C, although much more rapidly in the latter case. Data on amounts of gas extracted from small whole tubers indicate that the decrease in respiration rate as the maturity and storage periods advance partially results from the increase in development of the periderm as a barrier to the passage of oxygen and carbon dioxide.

White Rose tubers have a higher respiratory activity and a shorter rest period than Irish Cobbler tubers.

Treating tubers with ethylene chlorhydrin and keeping them in moist storage tend to abbreviate the rest period and cause a striking increase in rate of loss of carbon dioxide and absorption of oxygen over the chemically untreated, dry-stored tubers.

Analyses of interior gases in relation to the rate of carbon dioxide loss and oxygen absorption indicate that chemical treatments and moist storage facilitate the exchange of gases in potato tubers.

Tubers stored moist at 25° C in jars of air and of 60 per cent oxygen, absorbed much more oxygen than dry-stored tubers, but gave off only slightly more carbon dioxide during a 48-hour period. The presence of a solution of potassium hydroxide in the jars increased the absorption of oxygen by the tubers. Tubers in an atmosphere of 60 per cent oxygen absorbed slightly more of this gas than those in air. The relation of the composition of the interior gas to the above treatments also indicates that moist tubers are more permeable to carbon dioxide and oxygen than dry tubers.

Results of amounts of gas extracted from small, whole White Rose tubers indicate that the chemically treated and moist-stored tubers are more permeable to gas than the chemically untreated dry-stored tubers. All tubers become less permeable to gas as the period between harvest and extraction increases.

Comparisons of the amount and composition of the gas extracted from peeled and unpeeled whole tubers indicate that the periderm offers resistance to the passage of carbon dioxide and oxygen, also that the interior tissue of the tubers retards somewhat the passage of these gases.

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VACCINATION OF CALVES AGAINST TUBER- CULOSIS WITH CALMETTE-GUÉRIN CULTURE, BCG

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INTRODUCTION

When in June, 1924, Calmette and Guérin⁽²⁰⁾ first published their offer to furnish BCG cultures for experimental trial, the writers took under consideration plans for testing this method of vaccinating animals against tuberculosis. The matter rested until 1926, however, when Mr. H. D. Williamson of Napa, having read press notices of the French experiments, became interested in the possibility of controlling tuberculosis by means of this material in his dairy herd of about two hundred cows on Grisly Island in the Sacramento-San Joaquin Delta. Mr. Williamson urged that cultures be secured at once and furnished funds to pay for cablegrams and airmail postage. Plans were then developed for conducting formal investigations under controlled conditions at the California Agricultural Experiment Station, as well as for starting the work with the culture in the Williamson herd.

The following paper includes a summary of the experiments with the culture BCG by Calmette and Guérin and others, a report of the investigations with the culture to date at the California station, and an account of the attempt to control tuberculosis by means of culture BCG in a commercial dairy herd, together with summaries and conclusions drawn from the work in California.

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SUMMARY OF THE EXPERIMENTS OF CALMETTE AND GUÉRIN AND OTHERS

The experiments by Calmette and Guérin concerning the resistance to tuberculosis conferred upon young cattle by the bile-attenuated tuberculosis culture BCG (bacille bilié Calmette-Guérin) may be summarized as follows:

In 1911 Calmette and Guérin⁽¹⁵⁾ published the results of experiments in which 14 heifers, about 3 years of age, were given each month intravenous doses of 200 milligrams of BCG. After 6 months of such treatment, 9 became cachectic, but, on slaughter, no lesions of tuberculosis were found. Attempts were made to infect some of the remaining vaccinated heifers by injecting cultures of virulent bovine tubercle bacilli intravenously. It was determined by guinea-pig tests that these heifers excreted the virulent bacilli by way of the intestinal tract, but when they were slaughtered, no visible lesions of tuberculosis could be found.

In 1913 and 1914 Calmette and Guérin^(17, 18) reported that 7 heifers had been vaccinated intravenously with two doses of 1 and 5 mg, respectively, of the thirty-third and thirty-fourth transplants of BCG on ox-bile potato. Thirty days after the second vaccination, a dose of 3 mg of virulent tubercle bacilli was given intravenously to each of the 7 heifers and to 1 nonvaccinated control. The control died of miliary tuberculosis in 34 days. The vaccinated heifers remained apparently normal and were killed at 1, 2, 3, 4, 8, 12, and 18 months, respectively, after the test injection. No visible lesions of tuberculosis were found, but in all 7 cases virulent tubercle bacilli were demonstrated by guinea-pig inoculation in the apparently normal bronchial lymph nodes.

In 1920, Calmette and Guérin⁽¹⁹⁾ published the results of a carefully controlled trial, begun in 1912, of the resistance to tuberculosis of vaccinated heifers when associated with cows having open tuberculous lesions. Ten non-reacting heifers, aged 9 to 10 months, were selected, 4 of them being reserved as controls. Each of the other 6 received, in the jugular vein, 20 mg of bacilli from a 2-week-old planting of BCG, the seventieth transplant on ox-bile potato. On the same day, the 10 heifers were placed, together with 5 adult tuberculous cows, in a stable arranged to favor natural infection. The experiment lasted for 34 months, during which time some of the 5 tuberculous cows died and were immediately replaced by other open cases of

tuberculosis. After 18 months of such exposure to tuberculosis, none of the 6 vaccinated heifers reacted to tuberculin, while 3 of the controls gave definite reactions.

Three of the 6 vaccinated heifers, at the end of a year, received intravenously a second injection of 20 mg from a 21-day culture of BCG, the eighty-ninth transplant on ox-bile potato, and 2 of these 3 heifers received again, 12 months later, 20 mg of the one hundred and thirteenth generation of BCG culture. In March, 1915, 1 of these 2 heifers was killed accidentally. No lesions of tuberculosis could be found, and guinea pigs inoculated with portions of her bronchial lymph nodes were found normal on autopsy after 5 months.

On account of the war, it became necessary to terminate the experiment in August, 1915. On autopsy of the 9 remaining heifers, the following observations were made:

1. Three controls presented some tuberculous lesions, which were in general of slight extent. The fourth control, which had never reacted to tuberculin, was free from lesions.

2. Two of the 3 heifers vaccinated only in 1912 had a few small tubercles in the lungs and in the bronchial, mediastinal, and mesenteric lymph nodes. The third was free and portions of its lymphatic tissue inoculated into guinea pigs did not cause tuberculosis.

3. The heifer which had been vaccinated in 1912 and again in 1913 was free from tuberculous lesions, and portions of its lymphatics, injected into guinea pigs, did not cause tuberculosis.

4. The 2 heifers vaccinated in 1912, 1913, and 1914 were free, and portions of their lymphatics failed to produce tuberculosis in guinea pigs.

Calmette and Guérin concluded from this experiment that the intravenous injection of BCG not only confers upon healthy bovines a tolerance against artificial test inoculation, but also protects them for a time against infection from close and continued cohabitation in infected stables. They concluded that this tolerance, apparently linked with the presence of nonvirulent bacilli in the body, does not last longer than 18 months after a single vaccination. They stated that the tolerance could be maintained by vaccinations carried on each year.

In 1924, Calmette and Guérin⁽²⁰⁾ reported an experiment with 20 heifers, 7 to 8 months old and free from tuberculosis as indicated by the tuberculin test. Heifers 1 to 12 were vaccinated subcutaneously in the dewlap with BCG, the even numbers receiving 50 and the odd numbers 100 mg, respectively. Numbers 13 to 18 served as controls and were maintained under the same conditions. The 12 vaccinated heifers were divided into six groups of 2 animals each (1-2, 3-4, etc.).

With each group was placed one of the nonvaccinated control heifers, each group of three occupying an isolated stall. To test the resistance conferred by the vaccine, virulent bovine tubercle bacilli from cultures were weighed in a fresh state and inoculated into 19⁵ of the heifers, intravenously, in 5-mg doses with the following results:

Group I. Vaccinated heifers 1 and 2 and control No. 13 were inoculated 1 month after vaccination. Control No. 13, killed 60 days after inoculation, showed advanced miliary tuberculosis of the lungs. Vaccinated heifer No. 2 died of verminous bronchitis 5 months and 7 days after inoculation. No lesions resembling tuberculosis were found. Guinea pigs, inoculated with portions of the apparently normal left bronchial lymph node, contracted tuberculosis. Vaccinated heifer No. 1 remained in good condition and was butchered 12 months after inoculation. No lesions of tuberculosis could be found. Portions of the left bronchial lymph node were injected into 4 guinea pigs. These were killed but no indication of tuberculosis could be found in them.

Group II. Vaccinated heifers 3 and 4 and control No. 14 were inoculated 3 months after vaccination. Control No. 14 died of miliary tuberculosis 44 days after inoculation. Vaccinated heifers 3 and 4 remained in good condition. They were slaughtered 11 months after the test inoculation and no tuberculous lesions could be found. Portions of their bronchial lymph nodes were inoculated into guinea pigs. These pigs were killed 45 days after inoculation. Those that had received tissue from heifer No. 4 were tuberculous, but those inoculated from No. 3 were free from tuberculosis.

Calmette⁽¹²⁾ in the latest edition of his book *L'Infection Bacillaire et la tuberculose chez l'Homme et chez les Animaux* attributes great importance to the observation that the guinea pigs inoculated from heifers 1 and 3 remained free from tuberculosis. In previous experiments he had observed that calves vaccinated by the intravenous route and later inoculated by the same route with virulent bacilli, remained free from caseous lesions, but their apparently normal lymph-node tissue would retain the bacilli virulent for guinea pigs for at least 18 months. The fact that guinea pigs did not contract tuberculosis when injected with tissues from heifers 1 and 3 was interpreted by Calmette to indicate that the subcutaneous injection of BCG is more efficacious than the intravenous method of vaccination.

Group III. Vaccinated heifers Nos. 5 and 6 and control No. 15 were inoculated 6 months after vaccination. Control No. 15 became very emaciated, was killed 60 days after inoculation and was found to

⁵ Only 19 heifers are accounted for in their descriptions.

have extensive miliary tuberculosis of the lungs with some hepatization. Vaccinated heifers 5 and 6 were slaughtered 8 months after inoculation and no lesions of tuberculosis were found, but their apparently normal bronchial lymph nodes contained tubercle bacilli virulent for guinea pigs.

Group IV. Vaccinated heifers 7 and 8 and controls 16 and 16A were inoculated 12 months after vaccination. On account of the long delay, it was thought necessary to have 2 controls. The 2 controls died of miliary tuberculosis in 32 and 58 days, respectively. The vaccinated heifers Nos. 7 and 8, remained in excellent condition and were slaughtered 3 months after the infecting inoculation. No tuberculous lesions could be found, but their apparently normal bronchial lymph nodes contained tubercle bacilli virulent for guinea pigs.

Group V. Vaccinated heifer No. 10 died 25 days after vaccination. Vaccinated heifer No. 9 and control No. 17 were inoculated with virulent bacilli 15 months after vaccination. Control No. 17 died of miliary tuberculosis 54 days after inoculation. Vaccinated heifer No. 9 was butchered 2 months after inoculation. No lesions typical of tuberculosis were found, although the bronchial and mediastinal lymph nodes were reported by the veterinary director of the abattoir to be slightly enlarged and juicy. No mention was made of guinea-pig inoculations.

Group VI. Vaccinated heifers 11 and 12 and control No. 18 were inoculated 18 months after vaccination. The control died of miliary tuberculosis in 42 days. After the intravenous inoculation, with virulent tubercle bacilli, No. 11 gave the immediate thermal reaction typical in vaccinated calves, while No. 12 did not show this reaction but, on the 13th to the 18th days it showed a marked hyperthermia (39.8° C), after which it became apparently normal in every way. The 2 vaccinated heifers were killed 2 months after inoculation. Vaccinated heifer No. 11 was apparently free from tuberculosis, although its lymph nodes were somewhat enlarged and soft. In vaccinated heifer No. 12, the lungs were firm and incompletely collapsed. On section, the lung tissues were found thick and hard, but permeable throughout. The bronchial and mediastinal lymph nodes were firm and enlarged to twice normal size. On section, they were found to contain white islets, inclining to gray, surrounded by a zone mixed with blood, almost hemorrhagic, without visible tuberculous nodules. Calmette concluded that No. 12 had reached the limit of its tolerance after 18 months, although its resistance was still sufficient to prevent it from contracting the form of acute miliary tuberculosis shown by the control.

In 1927, Guérin, Richard, and Boissiere⁽³⁷⁾ reported an attempt, begun in 1921, to eliminate tuberculosis from an infected herd of 50 to 70 cattle at Gruville by vaccination of the young calves with BCG. The harmlessness of BCG for cattle was indicated by the fact that 30 animals, vaccinated in 1921-1924 and in several cases revaccinated for 3 or 4 consecutive years, were found, on slaughter for beef, to be free from tuberculous lesions. Guérin and his associates expressed a belief in the efficacy of the vaccine in preventing tuberculous infection in the young cattle associated with many tuberculous cows under conditions in which no sanitary precautions were taken to prevent the spread of infection. However, definite proof of this seems to be lacking, especially since no controls were used. No precautions appear to have been taken to protect the young calves from infection during the first few weeks of their lives and, in several instances, the first vaccination was delayed from 3 to 8 weeks, although in a recent publication, Calmette and Guérin⁽²¹⁾ have pointed out the importance of protecting all vaccinated calves in herds where cases of open tuberculosis exist by observance of the following precautions:

- (1) The first injection of BCG vaccine should be made as soon as possible after birth and not later than the 15th day.
- (2) The vaccinated subjects should be protected from abundant and repeated contamination by isolation and by feeding milk free from tubercle bacilli.

They further state that if calves receive the harmless bacillus-vaccine before exposure to virulent bacilli, they will be "premune."⁶ If, on the contrary, virulent organisms are already installed in the animal the vaccine cannot play any useful rôle.

Attempts to confirm the findings of Calmette and his associates have been reported by investigators in various countries. So far as the experiments on cattle are concerned, the results have been variable.

Sanz⁽⁶⁴⁾ has reported the vaccination of 841 head of cattle of all ages, 286 of which were in herds from which reactors to the tuberculin test were removed before starting to vaccinate. No injurious effect was observed to result from the vaccine.

Fénelon⁽²³⁾ has reported preliminary results of a field trial on two infected farms where all of the calves have been vaccinated since 1924 at 3 weeks to 1 month of age. One hundred and eleven vaccinated calves on these farms, at the time of publication, were reported to be

⁶ According to Calmette, the word "premunition" was first proposed in 1924 by Edm. Sergent and Donatien to designate a condition of protective latent infection, such as exists in certain protozoan diseases, particularly bovine piroplasmosis.

in excellent condition. No tuberculous lesions were found in 32 vaccinated calves that were butchered or that had died of intercurrent diseases.

The results of Ascoli and his associates⁽¹⁾ have apparently supported the claim that subcutaneous injections of BCG will protect against an intravenous dose of virulent tubercle bacilli that is fatal to nonvaccinated cattle.

A preliminary report of the Ukrainian Commission summarized by Tzeknovitzer⁽⁶⁹⁾ states that 12 calves and 2 colts were injected subcutaneously or intravenously with 76 to 200 mg of BCG. They all continued to develop in a normal fashion. One of the calves which had received 76 mg subcutaneously was killed 6 months later. Autopsy showed it to be free from tuberculosis, except that at the point of vaccination there was a circumscribed infiltration of the tissues with acid-fast organisms.

Eight months after vaccination, another calf which received 300 mg intravenously was killed. Lymph nodes in various parts of the body were enlarged, but no lesions characteristic of tuberculosis could be found. Guinea pigs inoculated from the lymph nodes remained normal.

In a communication in 1927 Tzeknovitzer⁽⁷⁰⁾ reported a trial with 13 vaccinated and 4 control calves. Six to 8 months after vaccination these were subjected to intravenous infection with the small dose of 0.005 mg of Vallée's strain of virulent tubercle bacilli. His statement of the results may be summarized as follows:

After the injection of the virulent Vallée culture the controls did not show a thermic reaction, but the vaccinated animals had a typical rise in temperature corresponding to a tuberculin reaction. All of the controls died in about 6 weeks of miliary tuberculosis. One of the vaccinated animals died 6 weeks after infection. No evidence of tuberculosis was found in it, but there was impaction of the stomachs and fatty degeneration of the liver. The other vaccinated animals developed normally. Two were butchered 3 months after the intravenous infection. Autopsy showed a nodular tuberculosis of moderate intensity in one animal and a similar, but milder, condition in the other. These lesions were not sufficient to have any clinical effect on the general condition of the animals. One of the vaccinated calves died from causes other than tuberculosis 3½ months after intravenous infection. In the left lung were 7 to 10 small isolated tubercles tending to calcification. Two more of the vaccinated calves were butchered 6 months after infection. One had several isolated tubercles on the pleura and a large number scattered through the parenchyma of the

lung, but it was stated that there was no evidence of a tendency of these lesions to spread. The other calf, killed at the same time, had several calcified nodules in the bronchial and mediastinal lymph nodes and also a few pulmonary nodules. There was a slight hyperplasia of the pleura.

Another vaccinated calf was butchered 8 months after infection and showed 3 tubercles in the lungs and 1 on the pericardium. There was a slight serous hyperplasia of the pleura.

Ten months after vaccination, another vaccinated calf was butchered. In the lower part of the lung was a caseous mass, the size of a hazel nut and 6 or 7 others, each the size of a pea. One tubercle was found in the apex of the left lung. The nature of the caseous foci had not been determined at the time of publication. There was a slight hyperplasia of the visceral and parietal pleura.

All of these cattle killed 6, 8, and 10 months after infection, were reported to be clinically in excellent general condition.

In the third report of the Ukrainian Commission, Tzeknovitzer^(71, 72) stated that 50 calves in all had been used in the experiments and that the vaccination of cattle confers in general in these animals a manifest resistance to experimental infection.

Lange and Lydtin^(44, 45) vaccinated calves in various ways and 3 to 3½ months later infected them with intravenous doses of 2 mg of the Vallée strain of virulent bovine tubercle bacilli with the results shown in table 1.

In a further vaccination trial, Lange and Lydtin⁽⁴⁵⁾ injected 4 calves intravenously with 20 mg of Vallée strain of virulent bovine tubercle bacilli, 6½ months after vaccination of 2 of them. The results of this trial are shown in table 2.

They concluded that their results stand in contradiction to the observations of Calmette and Tzeknovitzer, and suggested that, in recent years, there may have been a weakening in virulence of BCG, thereby resulting in a reduction in its immunizing power.

Watson, McIntosh, and Konst⁽⁷⁷⁾ have reported experiments with 14 calves that were placed with tuberculous cattle within the week following vaccination. On slaughter after 15 to 25 months these calves all showed tuberculous lesions, varying in the different animals from involvement of two groups of lymph nodes to extensive generalization. The control animals, 3 in number, also showed lesions. Their conclusion was that as far as a comparison is possible with these unequal numbers, there was nothing in favor of the vaccinated animals, nor any evidence to indicate a greater degree of resistance on their part to tuberculosis.

In addition, Watson⁽⁷⁶⁾ has reported results on 7 calves which were vaccinated twice at intervals of 12 to 14 months. These and 3 unvaccinated controls were all exposed to natural infection through milk and by cohabitation with tuberculous cattle. On slaughter at an age of 19 to 25 months tuberculous lesions were found in all 10. Watson stated that there was no definite evidence that the vaccinated were more resistant than the unvaccinated.

TABLE 1
RESULTS OF VARIOUS METHODS OF VACCINATION IN TESTS BY LANGE AND LYDTIN*

Calf No.	Vaccination	Death, days after infection	Autopsy
2	160 mg BCG subcutaneous	Died, 56 days	Miliary tuberculosis of lungs. Closely studded with miliary to linseed-sized nodules. Bronchial lymph nodes enlarged without macroscopically visible nodules. Very fatty liver. No other changes of significance.
5	160 mg BCG subcutaneous	Killed, 75 days	Lungs flecked with hardly visible spots. Microscopical accumulations of round and spindle cells, typical of tubercles. Bronchial nodes had no lesions. Kidneys had many typical tubercles, microscopic in size.
7	160 mg BCG subcutaneous	Killed, 75 days	Lung flecked with discrete linseed-sized nodules. Microscopically like No. 5. No other lesions of significance.
21	160 mg BCG subcutaneous	Killed, 78 days	In apical lobes of lungs, many linseed-sized tubercles with yellow centers. Many such nodules in bronchial and mediastinal nodes. Microscopic findings typical of tuberculosis. Kidneys, miliary nodules.
12	20 mg BCG intravenous	Killed, 74 days	No lesions found.
1	20 mg human strain of tubercle bacilli	Killed, 74 days	No lesions found.
20	20 mg human strain of tubercle bacilli	Died, 60 days	Lungs, many pea-sized caseous nodules and miliary tubercles. Arthritis of left tarsal joint. Not possible to determine which lesions were the result of the human bacilli and which the result of the bovine.
9	Control	Died, 10 days	Bronchopneumonia and miliary tuberculosis of lungs in first stages.
11	Control	Died, 25 days	Massive miliary tuberculosis of the lungs. Bronchial nodes enlarged without tubercles.
19	Control	Killed, 74 days	Flecking of the lungs as in cases 5 and 7 with linseed-sized nodules, microscopically typical of tubercles. One kidney tubercle.
22	Control	Killed, 78 days	Lungs, separated miliary nodules, microscopically typical of tuberculosis as in calf 7.

* Table taken from the article by Lange and Lydtin.⁴⁴

Rankin⁽⁶³⁾ after experimenting for 4 years and using about 250 calves reports that the vaccine was entirely harmless, although in most cases the calves reacted to the tuberculin test subsequent to vaccination. The calves which were vaccinated with BCG and immediately exposed to infection showed moderately increased resistance to tuberculosis over unvaccinated controls. Calves vaccinated with BCG and subsequently kept under sanitary conditions for a period in order to permit resistance to develop before exposure to infection, showed 80 per cent immunity as compared to 14 per cent for the controls. He reported that the tuberculous lesions found in the vaccinated calves were in general much less pronounced as well as less numerous than those in unvaccinated animals.

TABLE 2
FURTHER RESULTS OBTAINED BY LANGE AND LYDTIN*

Calf No.	Vaccination	Death, days after infection	Autopsy
13	100 mg BCG subcutaneous	Died, 25 days	
14	100 mg BCG subcutaneous	Died, 17 days	
23	Control	Died, 23 days	Severe miliary tuberculosis of the lungs and distinct kidney tubercles in all calves.
24	Control	Died, 23 days	

* Taken from the article by Lange and Lydtin.⁴⁵

King and Park⁽⁴⁰⁾ from observations on 46 calves, 45 of which were still living, concluded that 35 had developed tubercles, *at the point of vaccination at least*, as a result of the subcutaneous injection of 50 mg of BCG, since they became allergic within 12 weeks after vaccination, but 27 of 29 had lost skin hypersensitivity by the 32nd week. In one of these the reaction to tuberculin became doubtful on the 42nd week and was negative at 72 and at 90 weeks. This animal after being transferred to a farm for breeding purposes contracted tuberculosis of the lungs and bronchial and mediastinal nodes.

They have also concluded that 2 out of 8 calves after being fed on BCG showed evidence of protection, the test in one of these animals being the subcutaneous injection of 5.0 mg of bovine tubercle bacilli and in the other of 0.1 mg intravenously. In 5 calves that had been vaccinated subcutaneously and later subjected to the subcutaneous injection of bovine tubercle bacilli, they concluded that there was some evidence of immunity, since in 3 instances the lesion was limited to cold abscess

formation, in 1 there were tuberculous lesions in an adjacent lymph node, and in 1 no lesions were found, whereas in the controls lesions were found in the adjacent nodes in every case except 1, where a cold abscess was present.

In another group of 4 calves vaccinated subcutaneously, 0.1 mg of bovine bacilli intravenously did not produce lesions, while this dose, as well as 0.01 mg, produced lymph-node lesions in 4 control calves. In 2 vaccinated calves 1.0 mg of virulent bacilli produced lesions only in the broncho-mediastinal groups, while the same dose in 2 control calves produced lesions in the mesenteric nodes as well as in the bronchomediastinal. In a feeding infection experiment and also in trials with the subcutaneous injection of 0.01 to 0.10 mg of bovine tubercle bacilli by these writers, the results were inconclusive, since both the vaccinated and control animals failed on autopsy to show any lesions.

Larson and Evans⁽⁴⁶⁾ have reported the completion of an experiment begun in 1926 in Illinois with 60 head of cattle under farm conditions. Some of these were vaccinated annually by the subcutaneous injection of 100-mg doses of BCG; others received injections of killed tubercle bacilli suspended in sodium ricinoleate, and a third group were left untreated as controls. Six months after the first vaccination of the experimental animals some tuberculous cattle were turned into a 20-acre field with them. The experimental groups were depleted in various ways, unrelated to the experiment, and the experiment was terminated in 1928, at which time the cattle were slaughtered.

The published autopsy results were in general as follows: Of 7 calves treated with BCG, 4 had tuberculous lesions. Of 8 calves treated with killed bacilli, 2 had tuberculous lesions. Of 6 control calves, 2 had tuberculous lesions. The writers stated that this would seem to indicate that the Calmette method of vaccination had no value whatever in cattle.

Studies at the United States Bureau of Animal Industry Experiment Station have been reported by Schroeder and Crawford,⁽⁶⁵⁾ in which vaccinated cattle were exposed, with an equal number of controls, to tubercle bacilli by intravenous and subcutaneous injections, by feeding, and by contact with known tuberculous animals. In the group where exposure was made by feeding virulent tubercle bacilli, they reported more extensive lesions, as a whole, in the vaccinated than in the unvaccinated. In the group infected by injection or natural exposure, including 9 vaccinated cattle and an equal number of controls, it was reported that resistance to the localization and generalization of tuberculosis was slightly more manifest in the vac-

einated than in the unvaccinated animals, but in no group was there manifest an immunity as measured by the prevention of infection.

A large number of reports have been published on the effect of BCG on small laboratory animals. These have been thoroughly reviewed up to 1928 by Kraus⁽⁴¹⁾ and Lowenstein.⁽⁵⁰⁾ An excellent review of the veterinary aspects of the Calmette-Guérin method has been prepared by Zwick and Witte,⁽⁸⁰⁾ who claim that infection experiments are of little significance in determining the practical usefulness of an antituberculosis vaccine unless supplemented by trials under commercial farm conditions.

Such a trial has recently been reported by Brinet,⁽⁵⁾ who has used the vaccine during the past 6 years on about 300 calves in a dairy near Paris. The records showed that, since 1895, an annual average of 5 or 6 carcass condemnations, partial or total, had been made by inspectors at the time of slaughter of animals marketed from this farm, but that since January, 1926, none have been condemned or even reported to be tuberculous. Among the animals marketed since that date are 35 vaccinated cattle, aged at least 4 years. The owner was quoted as stating that since the vaccine had been used in his herd, the profit and loss sheet had been favorably influenced.

In a recent publication, Gerlach⁽³⁵⁾ emphasizes the harmlessness of BCG. He reports on the completed experiments and on those still in progress in which 702 guinea pigs and rabbits, 29 monkeys, 381 calves, 46 oxen, 3 dogs, and 2 cats each received 20 to 250 mg of BCG either by the digestive tract or by subcutaneous, intraperitoneal, intravenous, subdural, intracerebral, or intratesticular injection. No matter in what manner the BCG was given, the result was always a localized lesion which gradually healed.

The findings in 10 guinea pigs and 8 rabbits 6 months after 25 mg of BCG were injected intracerebrally led Gerlach to conclude that it is not possible for him to recognize in BCG any infectious character for the central nervous system. In presenting further evidence to support the harmlessness of BCG, he claims that the reinoculation of cultures obtained from inoculated animals failed to augment the virulence and that after successive passages through animals, the virulence is also not increased. He, therefore, considers BCG as a fixed virus.

Gerlach failed to find any increased pathogenicity in the dissociated S variant, as claimed by Petroff,⁽⁵⁹⁾ and he is not prepared to recognize in the S colony of Petroff or any other variant a degree of virulence different from the entire BCG culture. These latter conclusions were based on the injection of variants isolated by Gerlach and Gmeiner and also of variants obtained from Tzeknovitzer, Petroff, and from R.

Kraus. Guinea pigs and rabbits and 2 bovines inoculated in these experiments have failed, after 6 months, to show any apparent evidence of disease.

Gerlach further reports on the field vaccination of 371 new-born calves in badly infected tuberculous environments. Thus far, no tuberculous lesions have been found in a number of these animals slaughtered for various reasons, and those still living are reported to be developing normally.

EXPERIMENTAL METHODS AT THE CALIFORNIA STATION

Great care has been used to follow explicitly the directions given by Calmette⁽¹⁰⁾ in propagating the cultures and preparing the vaccine. The precaution of using separate laboratory equipment and of transferring the stock cultures to bile potato every tenth and eleventh transplant has been observed.

In the controlled experiments at Berkeley and Davis precautions have been taken to protect the calves from virulent infection from birth to vaccination and also during the interval between vaccination and the first intentional virulent infection. At least as many non-vaccinated checks as vaccinated animals have been maintained, and in one of the experiments additional checks treated with heat-killed tubercle bacilli or with nonvirulent strains of acid-fast bacilli were used.

In the experiments on the commercial dairy belonging to Mr. Williamson, only a few controls have been available, and protection of the calves from infection until 30 days after vaccination has not been possible.

Selection of the Calves.—The calves described in the artificial-infection experiments at Berkeley and Davis were of the Holstein-Friesian breed, either purebred or the offspring of grade cows and purebred bulls. They were born in the University Dairy Herd at Berkeley or selected from other herds free from tuberculosis as indicated by the tuberculin test. If selected from other tuberculosis-free herds, they were brought to the University Dairy at Berkeley before reaching 10 days of age and reared in an environment believed to be free from virulent bovine tubercle bacilli.

Use of the Tuberculin Test.—The question of using the tuberculin test was discussed with Calmette by one of the writers (Traum) in 1926. It was thought desirable that no tuberculin tests should be made on either vaccinated calves or nonvaccinated control calves after they

had been subjected to infection. After 2 years' experimenting without the use of tuberculin on any calves, it was decided to use the test on the nonvaccinated control animals just previous to subjecting them to infection, as an added precaution to insure their being free from tuberculosis. Hence, the control calves subjected to infection since January 1, 1928, have been in all cases tuberculin tested. Also certain calves 4 weeks or older were tested with tuberculin before being vaccinated. Results as shown in tables 7B to 11B fail to indicate that the use of the intradermic test had any effect on the experimental results.

The test as used at this station consists of a single intradermic injection into a subcaudal fold of 0.1 cc of United States Bureau of Animal Industry intradermic tuberculin, hereafter referred to as B. A. I. tuberculin. This dose is reported by Dorset⁷ to be approximately equivalent to 25 mg of O. T.

Continuing the policy of Calmette and Guérin, the International BCG Conference of October, 1928,⁽²⁷⁾ adopted the ruling: "In order not to modify the receptivity of the animals, the tuberculin test must not be given to any of the experimental animals, either before or during the course of the experiments." The writers at the California station are now of the opinion, however, that it is better experimental procedure to test control calves at the time they are weaned, such a test forming a valuable check on the effectiveness of the methods used to protect all the calves from tuberculous infection during the first 5 or 6 weeks of life. The value of the tuberculin test on calves as a check on the efficiency of the methods used to protect them from infection while being fed on pasteurized milk from tuberculous cows has been demonstrated in the process of eradicating tuberculosis from a dairy of several hundred head of cattle, according to observations by Haring and Traum.⁽²⁸⁾

Preparation of the Vaccine.—The original cultures of BCG used in the experiments at the California station were obtained by one of the writers (Traum) at the Pasteur Institute, Paris. Four culture tubes of glycerine potato, each labeled *BCG 8* were received by him on April 7, 1926, and were mailed to Berkeley, where they arrived on April 21, 1926. Two additional culture tubes labeled *BCG 1 V-27-26* and *BCG 321 V-30-26* were received on June 16, 1926, at Paris and brought to New York by Traum. These were mailed from New York, arriving in Berkeley on July 10, 1926. Transplants from all except one tube grew vigorously on potato, 5 per cent glycerine broth, Sauton's medium, and 5 per cent glycerine ox-bile potato.

⁷ Dorset, M. Personal communication. 1929.

Care has been taken to grow and prepare the vaccine and test it for pathogenicity on guinea pigs in exactly the way prescribed in directions received from Calmette.⁽¹⁰⁾ The stock cultures have been maintained in Roux tubes on potato with 5 per cent glycerine broth for a series of nine generations. The tenth and eleventh generations have been propagated on 5 per cent glycerinated ox-bile potato and then replanted on potato in glycerine broth for nine generations. Old, fully-ripened potatoes are used, since it has been observed that media made from new potatoes give only a feeble growth of BCG. Sauton's medium has been used for propagating some of the vaccine serials. A separate incubator planting room and equipment have been used exclusively for BCG culture and vaccine preparation. The vaccine has been made from cultures not less than 19 days nor more than 26 days old, except in the case of calves vaccinated in April-July, 1926, with material taken directly from the original cultures received from Calmette. When ready for use, each cubic centimeter of vaccine contained 10 mg of BCG bacilli (weight after removal of excess moisture by blotting with filter paper), suspended in a sterile diluent consisting of 100 parts of distilled water, 1 part of chemically pure glucose, and 1 part of chemically pure glycerine, as prescribed by Calmette.⁽¹⁰⁾

Method of Vaccination.—The subcutaneous injections of vaccine into calves have been made by passing an 18-gauge hypodermic needle through the skin of the dewlap in such a way that it passed close to the inner layers of the skin for about an inch. The point of the needle was held firmly against the inner surface of the skin while the 10 cc suspension of BCG was injected to infiltrate the inner layers of the skin as well as the subcutaneous tissue. The purpose of the injection against the skin was to obtain any possible intracutaneous as well as subcutaneous immunizing effect.

Autopsy Methods and Guinea-Pig Injections.—All of the cattle at the end of the experiment were slaughtered in small establishments where official inspection was maintained, except those which were showing terminal symptoms of tuberculosis. The latter were killed at a University laboratory and the carcasses taken to a fertilizer works.

The heads, hearts, lungs, livers, and intestines with all the attached lymph nodes were removed in containers to the laboratory. The superficial inguinal or supermammary, the prescapular, and the precrural lymph nodes were removed from the carcass and also taken to the laboratory. The anal, sacral, internal iliacs, sublumbar renal lymph nodes, and those on the stomach were carefully sliced and examined at the abattoir. In this manner all the lymph nodes and

organs with the exception of the brain and some of the deeper unimportant lymph nodes were carefully observed. In the laboratory the lymph nodes were removed and each before inoculation into the guinea pigs was immersed in boiling water from 10 to 12 seconds, placed in sterilized pans or Petri dishes, and sectioned. In a carcass where lesions were very small or entirely absent, the nodes were cut with sterilized scissors into pieces not more than 3 to 4 mm in thickness, several hours being consumed in examination and in obtaining material from each animal for injection. The procedure of immersing lymph nodes in boiling water insured against the introduction of tuberculous infection from the exterior and was far superior to searing with a hot spatula since all crevices and folded tissues could be more readily exposed to the heat, and, besides, any portion of lymph nodes so treated was available for inoculation.

In carcasses where macroscopic evidence of tuberculosis was not present a fair and large composite sample of the lymph nodes and organs was injected into guinea pigs.

The guinea pigs were usually killed between 60 and 90 days after inoculation except in those cases where discharging tuberculous ulcers had developed at the point of inoculation, in which case they were killed at an earlier date.

THE EFFECTS FROM INTRAVENOUS AND SUBCUTANEOUS INJECTION OF VIRULENT TUBERCLE BACILLI IN VACCINATED AND CONTROL CALVES

In table 3 is summarized the results following the *intravenous infection* of 4 calves that had been vaccinated with BCG and of 4 nonvaccinated controls, and the *subcutaneous infection* of 2 calves that had been vaccinated with BCG and of 2 nonvaccinated controls.

For the infection tests, use was made of the bovine tuberculosis culture 271.⁸ The surface growths from 34-day-old transplants were ground for 25 minutes in a mortar and suspended in physiological

⁸ The history of bovine tuberculosis culture 271 is as follows: On September 1, 1926, guinea pig 132 was injected intramuscularly with liquid obtained by grinding together tuberculous lesions from 17 cows killed at an abattoir in Berkeley. Guinea pig 132 died of generalized tuberculosis on September 19, 1926, and a portion of its spleen was inoculated into guinea pig 210, which died of generalized tuberculosis on November 5, 1926. The spleen of No. 210 was inoculated into guinea pig 271, which died of generalized tuberculosis on December 22, 1926. Cultures of tubercle bacilli were obtained from the tissues. These proved to be highly virulent for guinea pigs, rabbits, cattle, and swine.

saline solution so that each cubic centimeter contained 0.4 mg of bacilli. From direct microscopic counts it was estimated that each milligram contained about 70 million bacilli.

The 6 animals (Nos. 3, 4, 103, 104, *A* and *B*) listed in tables 3 and 4 that were subjected to infection on January 28, 1929, constitute an experiment planned after reading the statements of Lowenstein⁽⁵⁰⁾ and of Petroff, Branch, and Jennings.⁽⁶⁰⁾ The former had stated that the proper control for BCG vaccination experiments would be massive injections of killed tubercle bacilli. The latter investigators had claimed that BCG probably had little if any more immunizing power than killed tubercle bacilli. In the same articles, these authors advocated the inauguration of experiments on cattle to confirm or refute Calmette's claims.

The infection trials on calves 1 and 2 at the California station in August, 1927 (table 3) had already supported Calmette's finding that BCG vaccination would protect calves against the rapidly fatal effects of the intravenous introduction of virulent tubercle bacilli, but, on account of Petroff's⁽⁵⁸⁾ statements, it seemed desirable to test the effects of intravenous vaccination with BCG and, at the same time, compare it with the effects of a massive intravenous injection of killed tubercle bacilli.

The heat-killed bacilli were prepared from the virulent tuberculosis culture 271 by removing the surface growth from several tubes of Duval's serum egg. The excess moisture was removed by blotting the mass of bacilli between sterile filter paper before it was weighed. Four grams of the damp bacilli were then suspended in 25 cc of Calmette's diluent, placed in a sealed tube and submerged in a water bath at 60° C for 30 minutes. More diluent was then added so that each cubic centimeter contained 5.2 mg of bacilli. This suspension was immediately injected by way of the jugular vein, each calf receiving 192 cc of liquid containing, in all, about 1 gram of killed bacilli. No deleterious effects were observed to follow the injections of killed bacilli. Guinea pigs inoculated intramuscularly with 50-mg doses were found normal on autopsy after 5 months, while guinea pigs injected with one millionth of a milligram before heating developed generalized tuberculosis in a few weeks. In order to eliminate the possibility of confusing lesions produced by the BCG or killed bacilli with those produced by the subsequent intravenous injection of virulent bacilli, 2 calves, Nos. 94 and 96, were retained as noninfected controls (see footnote, table 4).

Extensive trials by the British Royal Commission on Tuberculosis, as reported by Cobbett,⁽²⁶⁾ indicate that, in calves, infection by the

subcutaneous route affords a very uniform and satisfactory method of testing the virulence of various strains of bovine tubercle bacilli and is also a delicate test of the variations in resistance to tuberculosis in individual animals. At the California station such a test of the variation in resistance between 2 vaccinated and 2 control calves has been carried out, with results as shown for calves 5, 6, 105, and 106 in table 3.

TABLE 3

RESULTS FROM INTRAVENOUS OR SUBCUTANEOUS INFECTION OF VACCINATED
AND CONTROL CALVES

Method of in- jecting viru- lent tu- ber- cle bacilli	No.	Born	BCG vaccination		Intervals in days			Remarks For detailed autopsies see pp. 325-332
			Point of injection	Amt. mg.	Birth to vaccination	Vaccination to infection	Infection to autopsy	
2 mg into jugular vein	1	1927 Mar. 22	Dewlap.....	100	8	140	138	Remained apparently normal until butchered. Apparently normal lymphatics caused tuberculosis in guinea pigs.
	2	Apr. 18	Dewlap.....	100	4	117	309	Good general condition when butchered. Scattered, moderately extensive tuberculous lesions on autopsy.
	101	Mar. 8	Control.....				22	Died of miliary tuberculosis.
	102	Apr. 15 1928	Control.....				20	Died of miliary tuberculosis.
	3	Aug. 8	Jugular vein.....	50	51	122	50	Died of tuberculous pneumonia.
	4	Aug. 23	Jugular vein.....	50	36	122	110	Good general condition when butchered. Lungs, thoracic nodes, and spleen studded with small tubercles.
	103	Aug. 28	Control.....				32	Died of miliary tuberculosis.
	104	Sept. 3	Control.....				27	Died of miliary tuberculosis.
50 mg under skin of neck	5	1927 Sept. 11	Dewlap.....	100	6	176	120	Remained in fair general condition until butchered. Moderate tuberculous lesions.
	6	Dec. 19	Dewlap.....	100	3	80	70	Remained in fair general condition until butchered. Moderate tuberculous lesions.
	105	Dec. 9	Control.....				68	Killed <i>in extremis</i> . Massive tuberculous lesions.
	106	Dec. 23	Control.....				71	Killed <i>in extremis</i> . Massive tuberculous lesions.

The results from the intrajugular injection of 2 mg of virulent tuberculosis culture 271 into the 2 calves that had been vaccinated by the intravenous injection of 1 gram of heat-killed tubercle bacilli are given in table 4.

TABLE 4
RESULTS FROM THE INTRAVENOUS INFECTON OF CALVES VACCINATED WITH
HEAT-KILLED TUBERCLE BACILLI

No.	Born	Intervals in days			Remarks
		Birth to vaccination	Vaccination to infection	Infection to autopsy	
A	Aug. 30, 1928.....	29	122	29	Died of miliary tuberculosis.
B	Aug. 31, 1928.....	28	122	30	Died of miliary tuberculosis.

NOTE.—Calves *A* and *B* were infected at the same time and in the same way as calves 3, 4, 103, and 104 (table 3). In addition to these controls, calf 94 vaccinated with 50 mg BCG intravenously and calf 96 vaccinated with 1 gram of heat-killed tubercle bacilli intravenously were protected from infection for 5 months and then slaughtered. No indications of tuberculosis were found in them on autopsy 150 days after vaccination.

The results on calves *A* and *B*, which had been vaccinated with heat-killed tubercle bacilli, support the findings of many investigators that the inoculation of dead bacilli in experimental animals yields little or no immunity to tuberculosis. Webb⁽⁷⁸⁾ recently reviewed this controversy and pointed out that a large majority of those who have investigated the subject are of the opinion that use of the living bacilli is necessary to develop an appreciable resistance to tuberculosis.

Case Histories of Calves Infected Intravenously.—Following are the case histories of the 4 calves vaccinated with BCG which were infected intravenously, as shown in table 3:

No. 1. Steer calf, ear tag 22 (see table 3) born on March 22, 1927, was vaccinated in the dewlap on March 30, 1927, with 100 mg BCG serial 27. This serial was made from a 20-day growth of the ninth transplant on potato of the culture received from Calmette on July 10, 1926. A round, hard thickening of the subcutaneous tissue of the dewlap developed, which measured 35 mm in diameter on May 23, 1927. It became impalpable a few weeks later. On August 17, 1927, 140 days after vaccination, this calf was injected in the right jugular vein with 2 mg of virulent bovine tubercle bacilli from Culture No. 271, suspended in 5 cc of physiological salt solution. The animal remained apparently normal and was in pasture with the tuberculous herd from August 17 to December 31. It was butchered on January 2, 1928, 138 days after intravenous infection.

Autopsy: The general condition was good. No macroscopic lesions of tuberculosis could be found, the lymph nodes, lungs, and other viscera being apparently normal. Stained smears from the lungs and various lymph nodes failed to show any acid-fast bacilli. Guinea pigs injected with submaxillary, atlantal, retropharyngeal, and mesenteric lymph nodes and spleen pulp failed to develop tuberculosis, while guinea pigs inoculated with a mixture of gastrohepatic, bronchial and mediastinal, and prerural and prescapular lymph nodes developed generalized tuberculosis.

Comment: The absence of macroscopic lesions but the presence in some of the apparently normal lymph nodes of virulent tubercle bacilli is in accordance with the publications of Calmette.

No. 2. Steer calf, ear tag 33 (see table 3), born on April 18, 1927, was vaccinated in the dewlap on April 22, 1927, with 100 mg BCG serial 35. This serial was made from a 23-day growth of the tenth transplant on potato of the culture received from Calmette on July 10, 1926.

A round, hard swelling developed at the point of injection in the dewlap, which was 40 mm in diameter on March 21, 1928.

On August 17, 1927, 117 days after vaccination, this calf was injected in the right jugular vein with 2 mg of virulent bovine tubercle bacilli from culture No. 271 in the same manner as calf No. 1. The animal remained apparently normal and was pastured with the tuberculous herd of calves until March 2, 1928, when it was placed in an isolated pen. At this time, two hard, round subcutaneous nodules, each about 20 mm in diameter, were observed on the side of the neck near the point of intravenous injection. On April 21, these had each increased to a diameter of about 40 mm. The anterior of these was in the center of the jugular groove at the point of intravenous injection, and the posterior nodule was 60 mm anterior and 40 mm above the upper end of the left prescapular lymph node. On April 9, 1928, the posterior nodule was removed surgically and was found to be a thin, tough-walled abscess, containing soft, buff-colored glutinous pus which, on microscopic examination, was seen to consist of amorphous cell detritus. The macroscopic appearance of the abscess was more typical of pyogenic than of tuberculous infection. However, numerous acid-fast bacilli were found in smears. Inoculation of the pus, on April 9, 1928, resulted in generalized tuberculosis in 3 guinea pigs and a rabbit.

The surgical wound in the neck of the calf healed completely by granulation, and only a slight scar was visible after 6 weeks. The anterior nodule on the neck was left undisturbed, but it did not appear to grow any after April 9.

The calf remained in good general condition and was butchered on June 21, 1928, 309 days after its intravenous infection.

Autopsy: The general condition was good. A thickening was palpable through the skin, 50 mm above the superior end of the left prescapular lymph node. On section, a dirty gray homogenous connective tissue formation, somewhat resembling lymph-node tissue, was observed involving the inner layers of the skin, the subcutaneous tissue, and the muscle. The neoplasm measured 40 x 30 x 20 mm. Portions of this lesion were injected into guinea pig No. 616, which was gassed on August 20, 1928, and was found to be negative for tuberculosis.

In the left jugular furrow, near the point of intravenous inoculation, was a round, smooth, subcutaneous abscess, 40 mm in diameter, consisting of a firm connective tissue wall, 5 mm thick. The pus content was viscid, yellowish-green, and odorless. Acid-fast bacilli were demonstrated in smears from this pus.

In the subcutaneous tissue between the left ischial tuberosity and the anus was a round, smooth, hard abscess, 50 mm in diameter, similar in structure to the abscess in the jugular furrow. Portions of this lesion were injected into guinea pig No. 619, which was gassed on July 23, 1928, and showed generalized tuberculosis.

An area of chronic bronchopneumonia, 50 mm in diameter, was present in the posterior tip of each diaphragmatic lobe of the lung. Large numbers of lung worms (*Dictyocaulus viviparus*) were seen in the adjacent bronchial tubes.

A portion of the apparently normal spleen was injected into guinea pig No. 615, which was gassed on August 20, 1928, and found to be negative for tuberculosis.

The left bronchial lymph node contained a caseous area, 5 mm in diameter. The cut surface of the right bronchial lymph node showed a red area, 6 mm in diameter. The 2 bronchial lesions and portions of the apparently normal mediastinal lymph nodes were injected into guinea pig No. 620, which was gassed on July 23, 1928, and showed generalized tuberculosis.

In the center of the mesenteric chain of lymph nodes were 2 caseous areas, about 5 mm in diameter, each surrounded by a thin connective tissue wall. Acid-fast bacilli were abundant in smears. Portions of these mesenteric lesions were injected into guinea pig No. 618, which was gassed on August 16, 1928, and showed generalized tuberculosis. Adjacent to the gastrohepatic lymph nodes was an abscess, 30 x 20 x 20 mm, containing inspissated pus. This pus was injected into guinea pig No. 617, which was gassed on August 16, 1928, and showed generalized tuberculosis.

The well-marked tuberculous lesions observed in vaccinated calf No. 2 are similar to the cases described by Tzeknovitzer^(71, 72) in the Third Report of the Ukrainian Commission and indicate that some modification of Calmette's general conclusions is justified. It is noteworthy that tuberculous abscesses appeared in this calf about 7 months after intravenous infection and, on autopsy 10 months after infection, well-marked tuberculous lesions were visible in various parts of the body. The location and appearance of these, together with their high virulence for guinea pigs and a rabbit, justified the conclusion that the virulent bacilli injected intravenously on August 17, 1927, had remained alive in the tissues and after a time had resulted in the production of well-marked lesions. Those on the neck probably resulted from a few bacilli lodged in the subcutaneous and intramuscular tissue when the needle was being withdrawn from the jugular vein after the intravenous injection on August 17, 1927, but these lesions did not become palpable until March, 1928. Visible evidence was obtained that they were gradually increasing in size between March 2 and April 7. After the surgical removal of one of these, no increase in size was noted in the remaining nodule.

Calf No. 2 was associated at pasture with other tuberculous cattle from August 17, 1927, to March 2, 1928, and it is not certain that all of its tuberculous lesions were the result of intravenous infection. The caseation in the mesenteric and bronchial lymph nodes may have resulted either from tubercle bacilli ingested with the food or from the intravenous injection. The tuberculous abscesses in the ischial and gastrohepatic regions were unusual in location and appearance and are believed to have resulted from the intravenous injection of virulent bacilli.

No. 3. Steer calf, ear tag 92 (see table 3), born August 8, 1928, at Millbrae and brought to Berkeley September 20, was negative to intradermic tuberculin test given September 21, and was injected in the left jugular vein with 50 mg of BCG suspended in 2.5 cc of diluent on September 28. The animal remained apparently normal except for irregularities in temperature, which during the 2 weeks following the injection ranged from 102° to 105° F. Similar fluctuations in temperature also occurred in nonvaccinated control calves kept with this animal. The calf grew normally and on January 28, 1929, was subjected to infection by injection in the left jugular of 2 mg of virulent bovine tubercle bacilli from culture 271. The animal remained normal in general appearance until February 19, after which time it developed a chronic cough, showed some anorexia and marked hyperthermia. It gradually became weaker and died on March 19, 1929.

Autopsy: The carcass was greatly emaciated. Muscular tissues shrunken. Intermuscular tissue was edematous. The lungs were studded with gray-white nodules, varying from 3 to 12 mm in size. The lungs felt like a bag full of peas. In many places the diaphragmatic lobes were so thickly studded that the nodules were confluent, and when small chunks of these lobes were dropped into water, they immediately sank. The tips of the apical and cardiae lobes were not so thickly studded, there being spaces of normal tissue visible. Some of the larger nodules had caseous centers. The pulmonary lymph nodes were enlarged about five times and showed necrotic foci involving most of their tissue. There was considerable hyperplasia of the costal pleura. Other than this, the tissues were apparently normal, except for post-mortem changes. Acid-fast bacilli were abundant in the thoracic lesions, but none could be found in the apparently normal abdominal organs.

The chief difference between the lesions in No. 3 and in other animals of this group which had died previously was as follows:

The nonvaccinated controls and the 2 calves treated with heat-killed bacilli died of acute miliary tuberculosis, particularly of the lungs, but also involving organs in the abdominal cavity. Calf No. 3 appeared to have died of tuberculous pneumonia, the lesions being confined to the thorax. The tubercles, while very numerous in the lungs, were not as numerous as in the nonvaccinated calves. However, in calf No. 3 the tubercles were 3 to 12 mm in size, while in the control calves and in calves A and B they were all miliary in type, 2 to 3 mm.

No. 4. Steer calf, ear tag 94 (see table 3), born August 23, 1928, at Millbrae and brought to Berkeley September 20, was negative to intradermic tuberculin test given September 21 and was injected in the left jugular vein with 50 mg of BCG suspended in 2.5 cc of diluent on September 28. The animal remained apparently normal except for slight irregularities in temperature. On January 28, 1929, this calf received intravenously 2 mg of virulent tubercle bacilli from culture 271 in the same manner as calf No. 1.

During the 3rd and 4th weeks following, the animal had a persistent cough, rapid respiration, and occasionally slight discharge of blood from the nostrils. Its temperature showed relatively slight variations and it remained otherwise in apparently normal condition until slaughtered on May 18, 1929.

Autopsy: The carcass was well nourished. The lungs were sprinkled with yellow nodules 2 to 4 mm in diameter. The thoracic lymph nodes were enlarged three to five times and thickly studded with hard necrotic nodules. The spleen was studded throughout with white nodules 1 to 2 mm in diameter. The average distance between the nodules was about 30 mm. Acid-fast bacilli were demonstrated in the lesions indicated above.

Case Histories of Calves Infected Subcutaneously.—Following are the case histories of the 4 calves which were infected subcutaneously as shown in table 3:

No. 5. Steer calf, ear tag 69 (see table 3), born on September 11, 1927, was vaccinated in the dewlap on September 17, 1927, with 100 mg BCG, serial No. 62. It was reared in an environment believed to be free from bovine tubercle bacilli

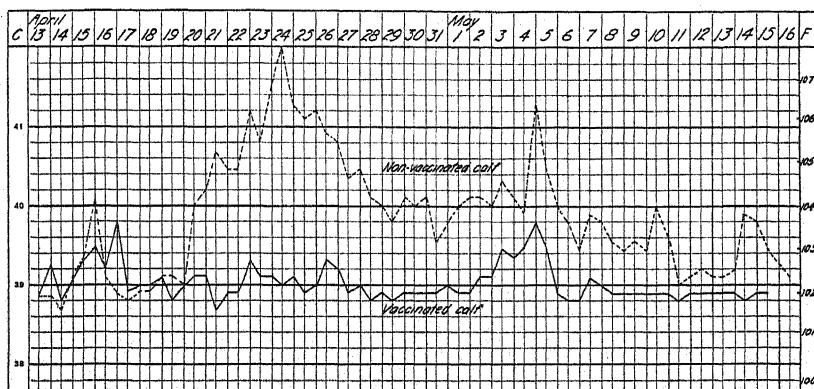


Fig. 1. Temperatures of calves Nos. 5 (vaccinated) and 105 (control), injected subcutaneously with 50 mg of virulent tubercle bacilli, March 12, 1928. The dotted line indicates the temperature of the nonvaccinated control.

until March 12, 1928. One hundred and seventy-seven days after vaccination, the animal was injected under the skin of the neck on the left side with 50 mg of virulent bovine tubercle bacilli from culture No. 271. (See page 322 for description of this culture.)

The animal remained apparently normal (fig. 1) except for the swelling at the point of inoculation which gradually developed and which on May 6, 1928, measured 90 x 50 x 35 mm. After this no increase in size was apparent (see fig. 4, p. 386). The calf was killed on July 10, 1928, 120 days after infection, in order to compare the autopsy findings with control animal 105.

Autopsy: The general condition was good. At the point of inoculation of the virulent bacilli was a nodule 70 x 35 x 20 mm, occupying subcutaneous tissue only and containing creamy tenacious pus. Adjacent and located 10 cm apart between the superficial neck muscles, were two abscesses well encapsulated, 20 x 10 mm, containing greenish-yellow granular pus. Nearby was an abscess similar in character measuring 10 x 10 mm.

A 120-mm linear abscess within the neck muscle tissue extended from the point of injection to another subcutaneous nodule, which was irregular in shape, and about 50 x 50 mm. The wall of this linear abscess was 30 mm thick.

The left prescapular lymph node was slightly enlarged and studded throughout with caseated (mostly soft) areas, varying in diameter from 3 to 10 mm. In the left bronchial lymph node was a necrotic point 0.5 mm in size. In the anterior mediastinal lymph node were two 2-mm nodules. A microscopic examination showed numerous acid-fast organisms in the abscesses in the neck. An occasional acid-fast organism was seen in smears from the left prescapular lymph node. Guinea pigs were injected as follows:

Source of material	Guinea pig No.	Date killed 1928	Results
Head and mesenteric nodes.....	667	Sept. 8	Negative
Lung and thoracic nodes.....	668	Aug. 13	Moderate tuberculosis
Muscle at point of injection.....	669	Aug. 13	Generalized tuberculosis

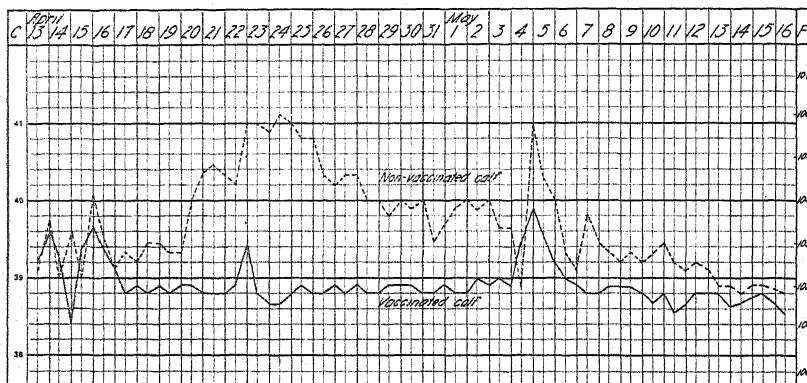


Fig. 2. Temperatures of calves Nos. 6 (vaccinated) and 106 (control), injected subcutaneously with 50 mg virulent tubercle bacilli, March 12, 1928. The dotted line indicates the temperature of the nonvaccinated control.

No. 6. Steer calf, ear tag 75 (see table 3), born on December 19, 1927, was vaccinated in the dewlap on December 22, 1927, with 100 mg BCG. It was reared in an environment believed to be free from virulent tubercle bacilli until March 12, 1928, 81 days after vaccination, when it was injected subcutaneously in the left side of the neck with 50 mg of virulent tubercle bacilli from culture No. 271. The animal remained apparently normal except for a subcutaneous swelling at the point of injection, which measured 90 x 50 x 35 mm on May 6, 1928. (See fig. 5.) The temperature remained fairly normal (fig. 2). Slaughtered on May 21, 1928, 70 days after infection, for the purpose of comparing with steers 105 and 106.

Autopsy: The general condition was good. In the subcutaneous tissue of the neck, at the point where virulent tubercle bacilli had been injected, was a flat, thin-walled abscess, 100 x 50 x 35 mm, containing soft, creamy pus. Smears showed an occasional acid-fast bacillus. The adjacent prescapular lymph node was slightly enlarged (size 100 x 35 x 35 mm) and its cut surface was studded with calcareo-caseous nodules, 1 to 5 mm in diameter. Several nodules each about 2 mm in diameter were found in the parenchyma of the spleen, and guinea pig No. 607 was

inoculated with some of these nodules. It was gassed on July 12, 1928, and found to have generalized tuberculosis.

The lymph nodes, other than the left prescapular, were apparently normal throughout the viscera and body. Bronchial and mediastinal lymph nodes were injected into guinea pig No. 606. It was gassed on July 12, 1928, and showed generalized tuberculosis. Portions of the apparently normal mesenteric lymph nodes were injected into guinea pig No. 604, and the lung tissue into No. 605. They were gassed on July 17, 1928, and both found free from tuberculosis.

No. 105. Steer calf, ear tag 191 (see table 3), born on December 9, 1927, was reared on an environment believed to have been free from virulent bovine tubercle bacilli. It was negative to an intradermic test with 0.2 cc B. A. I. tuberculin on March 1, 1928. It was used as a nonvaccinated control for steers 5 and 6, by the subcutaneous injection, on March 12, 1928, of 50 mg of virulent bovine tubercle bacilli from culture No. 271. The animal developed a swelling at the point of injection, which rapidly increased in size until May 6, 1928, at which time it measured 160 x 120 x 70 mm, and the adjacent prescapular lymph node was 230 x 130 x 120 mm (see fig. 6). After this date these swelling increased slowly. On April 20, the animal's temperature rose to 104° F and fluctuated between 104° and 106° F for a week, after which it gradually returned to normal by May 6 (fig. 1).

The animal was depressed in appetite and activity during the period of high temperature. About May 1, the abscess in the side of the neck ruptured through the skin and continued to discharge until date of slaughter. The animal appeared to be in such an advanced stage of tuberculosis that it was decided to slaughter it on May 19, 1928, 68 days after infection.

Autopsy: The general condition was fair, except for the massive lesions on the left side of the neck. The abscess at the point of injection consisted of a mass of caseo-calcareous tuberculous tissue, 200 x 190 x 80 mm, in the center of which was a spot of soft, putrid, caseous liquefaction, 50 x 40 x 40 mm. An edematous mass of loose connective tissue surrounded the left prescapular lymph node. Including the node, it measured 230 x 130 x 120 mm. The lymph node itself was enlarged and changed into a tuberculous mass, 190 x 110 x 110 mm, in the center of which was a spot of soft, caseous liquefaction, 40 mm in diameter.

The lungs, liver, and spleen were studded throughout with grey nodules, ranging from 2 to 10 mm in diameter. The distance between the tubercles in the lung ranged from 5 to 20 mm. Eighty-one such nodules were observed on the diaphragmatic surface of the liver, which illustrates about the density of those throughout the liver and spleen.

The thoracic, axillary, pectoral, and left radiohumeral lymph nodes were enlarged several times and their cut surfaces were thickly studded with caseo-calcareous nodules. Each of the cervical and gastrohepatic lymph nodes contained several caseous nodules, and a caseous nodule was found in one of the lymph nodes of the mesenteric chain.

A number of pendulous nodules, ranging from 5 to 10 mm in diameter, were observed on the omentum and serous surfaces of the mesentery and intestines. Some of these nodules had caseous centers. Acid-fast bacilli were demonstrated in smears from various lesions, including the lungs, kidney, and the one mesenteric node mentioned.

No. 106 Steer calf, ear tag 193 (see table 3), born on December 23, 1927, was reared in an environment believed to have been free from virulent bovine tubercle

bacilli. It was negative to an intradermic test with 0.2 cc B. A. I. tuberculin on March 1, 1928. It was used as a nonvaccinated control for steers 5 and 6 on March 12, 1928, by the subcutaneous injection of 50 mg of virulent bovine tubercle bacilli from culture No. 271.

The animal developed a swelling at the point of injection in the left side of the neck, which rapidly increased in size until May 6, 1928, at which time it measured 180 x 120 x 80 mm, and the adjacent prescapular lymph node was 200 x 100 x 100 mm (see fig. 7). After that date, these swellings increased slowly. On April 20 the animal's temperature rose to 104.0° F and fluctuated between 104.0° and 107.6° F for 10 days, after which it gradually returned to normal, which it reached by May 6 (fig. 2). It was depressed in appetite and activity during the period of high temperature. The animal developed such an advanced stage of tuberculosis that it was killed on May 22, 1928.

Autopsy: The general condition was fair. In the subcutaneous tissue of the neck, at the point where virulent tubercle bacilli had been injected, was a solid caseocalcareous mass, 185 x 150 x 90 mm. The adjacent prescapular lymph node apparently measured 210 x 110 x 100 mm, but when cut across, it was noted to be 100 x 70 x 70 mm, and the balance of the swelling was edematous connective tissue. The cut surface of the lymph node was caseocalcareous and firm.

The liver, lungs, and spleen were studded throughout with white and pinkish-white nodules, ranging from 1 to 5 mm in diameter. Two hundred and fifty such nodules were visible on the diaphragmatic surface of the liver. Acid-fast bacilli were abundant in the neck lesion and liver lesions. Five pendulous nodules, each 5 mm in diameter, were observed on the surface of the right costal pleura.

The omentum and mesentery were studded with pendulous pinkish-white nodules, about 5 mm in diameter. It was estimated that there was one for each square decimeter of surface. The bronchial, mediastinal, and gastrohepatic groups of lymph nodes were enlarged five to ten times and their cut surfaces were thickly studded with caseous nodules, 1 to 3 mm in diameter. The following groups of lymph nodes were slightly enlarged and each node contained one to seven caseous nodules, from 1 to 3 mm in diameter: the submaxillary, atlantal, retropharyngeal, left axillary, left scapulohumeral, and both prercurals. Smears from the retropharyngeal lymph nodes showed acid-fast bacilli. About one-third of the nodes of the mesenteric chain showed on their cut surfaces 2 to 5 caseous nodules ranging from 1 to 5 mm in diameter.

Some of the spleen nodules were injected into guinea pig No. 608, which was gassed on July 12, 1928 and showed generalized tuberculosis.

In the 4 calves (Nos. 5, 6, 105 and 106) that were infected subcutaneously it was noted that the most rapid development of the local lesions occurred during the first 3 weeks following the subcutaneous infection and that during this time hyperthermia and anorexia occurred in the controls. The controls showed a distinctly greater susceptibility as evidenced by the temperatures shown in figures 1 and 2, the general symptoms of the animals, and particularly the tuberculous lesions found on autopsy. It is believed that the 2 controls would have died of tuberculosis in a few days if they had not been slaughtered for humane reasons. On the other hand, the lesions

in the 2 vaccinated calves were so limited in extent that it appears probable that they would have lived indefinitely.

Discussion of the Results of Intravenous and Subcutaneous Infection as Shown in Tables 3 and 4.—The relatively high virulence of test culture No. 271 for cattle was demonstrated by the death from miliary tuberculosis of the 4 nonvaccinated control calves and the 2 calves treated with killed tubercle bacilli in 20 to 32 days, after intravenous infection with a 2-mg dose. The appearance of the tissues of these calves were all about the same. The lungs of each were filled with grey nodules 1 to 4 mm in size and there was a sprinkling of similar nodules throughout the liver and kidneys. Acid-fast bacilli were demonstrated in smears and in sections and the macroscopic and microscopic findings were typical of acute miliary tuberculosis. There was no distinguishable difference between the lesions of the nonvaccinated controls and those which had received the heat-killed tubercle bacilli.

On account of need for brevity a detailed description and history of the control calves infected intravenously has been omitted from this paper.

The fact that the 2 calves (Nos. 1 and 2) which had been vaccinated in the dewlap lived on for several months after intravenous infection without showing any outward evidence of tuberculosis, while the 2 controls (Nos. 101 and 102) died of miliary tuberculosis in 20 and 22 days respectively is in accordance with the results of experiments by Calmette and Guérin.⁽²⁰⁾ Also, the fact that calves (Nos. 3 and 4) that had been vaccinated intravenously with BCG both showed clinical symptoms within a month after infection supports the claims of Calmette that subcutaneous vaccination with BCG is more effective than intravenous vaccination in creating resistance to tuberculosis.

The lesions found at autopsies of Nos. 3 and 4 indicated that these calves were more resistant than the controls, and the fact that they lived longer than the controls would also show that some resistance had been produced by the BCG vaccine.

The injurious effect of the virulent bacilli on the controls Nos. 105 and 106 contrasted with the distinctly less marked pathologic effect on 2 vaccinated calves Nos. 5 and 6, indicates that the BCG had conferred resistance to subcutaneous infection. This experiment acquires added significance when compared with the results obtained in the calves infected in other ways, as described later in this paper.

Cobbett⁽²⁶⁾ summarized the data of the British Royal Commission and the German Gesundheitsamt and called attention to the marked

differences in the power to resist tuberculous infection which occur in individual calves, showing that such differences play a more important part when virulent bacilli in doses of moderate size (10 mg) are injected subcutaneously than when larger doses (50 mg) are given in the same manner. According to Cobbett the workers in the German Gesundheitsamt noted variations in susceptibility to tuberculosis among calves, and they recommend that when but a single calf is used in typing a strain and unexpected results are obtained, the experiment should be repeated on another calf.

THE EFFECTS OF FEEDING TUBERCULOUS TISSUES AND MILK TO VACCINATED AND CONTROL CALVES

After weighing carefully the arguments for and against natural and artificial infection in an effort to determine the protective efficiency of the BCG culture, it was decided that much more can be learned from artificial feeding infection with tuberculous tissues than by the somewhat haphazard exposure to infected cattle or environs, and since the vaccination trials in the Williamson herd involved natural infection, efforts at Berkeley and Davis were concentrated on artificial infection with tuberculous material.

Fifty vaccinated calves and an equal number of unvaccinated controls have been subjected to infection by feeding tuberculous material. The tissues were ground in a meat-grinding machine, thoroughly mixed with milk, and except that fed to animals in table 6, was strained through several layers of cheese cloth. Each dose was fed separately to each calf in the manner described in the footnotes to the tables. Animals listed in table 6 were given the ground, unstrained tuberculous tissue in milk.

The tissues were obtained from cattle condemned at an abattoir or from guinea pigs and rabbits inoculated with bovine tuberculous materials. In addition, 4 vaccinated calves and 4 controls were fed on milk from the tuberculous udders of cows No. 600 or No. 2171 (see figs. 9 and 10).

The sources of the tuberculous tissue, the respective amounts fed each of the 100 calves, and the results of the microscopic and biologic control tests on each feeding are given in table 5.

Before giving the first infecting feeding, the vaccinated calves and the nonvaccinated controls, which had been reared together at the dairy in Berkeley in an environment protected as much as possible from virulent tubercle bacilli, were transferred to an isolated barn

TABLE 5

SOURCES AND AMOUNTS OF TUBERCULOSIS TISSUES AND BACILLI USED TO INFECT CALVES BY FEEDING

Label numbers of calves fed	Date collected	Description of material	Date fed	Dose fed each calf		Guinea-pig control	
				Grams of tissue*	Estimated acid-fast bacilli†	Mg. tissue injected‡	Result
	1926 Sept. 1	Portions of tuberculous lymph nodes, lungs, and livers of 17 cattle, condemned at an abattoir.....	1926 Sept. 2	80	\$	\$	+
7-10, and 107-110	Sept. 2	Tissues from a guinea pig injected with bovine lesions.....	Sept. 6	6	\$	\$	+
	Sept. 26	Tissues from guinea pigs injected with bovine lesions.....	Sept. 27	4	\$	\$	+
	Oct. 23	Active tubercles from the pleura, spleen, and omentum of a cow.....	Oct. 25	37	\$	500.0	+
7-10, 107-110, C, D, E, and F	Nov. 26	Caseous pus from tuberculous cattle tissues condemned at an abattoir....	Nov. 27 Nov. 29	80 80	\$ \$	250.0 25.0	++
	1927 Aug. 12	Tuberculous tissue from the lungs, costal pleura and lymph nodes of 2 aged cows and one 7-month calf.....	1927 Aug. 13 Aug. 15	10 10	500,000 500,000	50.0 50.0	++
11-44 111-144	Aug. 16	Tuberculous tissue from the lungs, costal pleura, and lymph nodes of an aged cow.....	Aug. 17 Aug. 19 Aug. 22 Aug. 23	2 2 2 2	690,000 690,000 690,000 690,000	0.5 1.25 1.25 1.0	++
	Aug. 26	Tuberculous tissue from 2 rabbits and 2 guinea pigs.....	Aug. 27	2	1,320,000	1.0	+
	Sept. 1	Tissues from lungs and lymph nodes of a range steer.....	Sept. 2	2	850,000	1.0	+
	Sept. 7	Lung of calf No. 101, which died of miliary tuberculosis (see table 3).....	Sept. 8 Sept. 9	0.5 0.5	1,890,000 1,890,000	0.5 0.5	++
	Sept. 10	Lymph node tissue, calf No. 101.....	Sept. 11 Sept. 12	2 2	960,000 960,000	2.0 2.0	++
30-44 and 130-144	Sept. 13	Lung of calf No. 102, died of miliary tuberculosis, (see table 3).....	Sept. 14 Sept. 16	5 5	1,950,000 1,950,000	5.0 5.0	++
	Sept. 13	Lung and lymph nodes, calf No. 102.....	Sept. 18 Sept. 19	0.3 0.3	2,000,000 2,000,000	0.3 0.3	++

TABLE 5—(Continued)

Label numbers of calves fed	Date collected	Description of material	Date fed	Dose fed each calf		Guinea-pig control	
				Grams of tissue*	Estimated acid-fast bacilli†	Mg tissue in- jected‡	Re- sult
30-44 and 130-144 (Cont.)	Sept. 28	Tuberculous viscera from guinea pigs that had been inoculated with the feeding, August 13, this table.....	Sept. 24	1.0	2,040,000	1.0	+
			Sept. 28	0.5	1,020,000	0.5	+
			Sept. 29	0.5	5,850,000	0.2	+
			Sept. 30	0.5	5,850,000	1.0	+
			Oct. 1	0.5	5,850,000	1.0	+
			Oct. 2	0.5	5,850,000	0.1	+
			Oct. 3	0.5	5,850,000	0.2	+
			Oct. 4	0.5	5,850,000	0.1	+
			Oct. 5	1.11	12,980,000	5.5	+
			Oct. 6	1.11	12,980,000	5.5	+
40-44 and 140-144	Oct. 10	Caseous material from tuberculous lesions in the lungs and lymph nodes of an aged cow.....	Oct. 7	1.11	12,980,000	0.5	+
			Oct. 8	1.11	12,980,000	0.005	+
			Oct. 10	Tuberculous viscera of 14 guinea pigs inoculated from feedings, Sept. 2-8, this table.....	Oct. 11	1.11	12,980,000
			Oct. 12		Oct. 11	1.11	0.1
					Oct. 12	1.11	2.0
			Oct. 13	Tuberculous viscera of 8 guinea pigs inoculated with bovine lesions.....	Oct. 14	1.8	45,000,000
			Oct. 15		Oct. 15	1.8	45,000,000
			Oct. 16		Oct. 16	4.8	67,500,000
			Oct. 17		Oct. 17	4.8	67,500,000
			Oct. 18	Tuberculous viscera of 21 guinea pigs inoculated with feedings, Sept. 14-19, this table.....	Oct. 19	6.8	0.000034
49-54 and 149-154	July 6	Viscera of 3 guinea pigs and 1 rabbit which had died of tuberculosis after injection with bovine lesions.....	Oct. 19			73,100,000	—
			Oct. 20	11.0	Oct. 20	3,500,000,000	0.000005
					1928		+
			July 7	42.0	July 7	210,000,000	0.00001
55-59 and 155-159	July 6	Caseous and cellular tissue from tuberculous lesions in the lungs and thoracic lymph nodes of 2 cows condemned at an abattoir.....	July 9	14.0	July 7	70,000,000	1.0
			July 11	14.0	July 9	70,000,000	1.0
					July 11	70,000,000	0.00001

TABLE 5—(Concluded)

Label numbers of calves fed	Date collected	Description of material	Date fed	Dose fed each calf		Guinea-pig control	
				Grams of tissue*	Estimated acid-fast bacilli†	Mg tissue injected‡	Result
60 and 160	Aug. 8	Caseous and cellular material from tuberculous lesions in the costal pleura or in the lungs and lymph nodes of a steer, a cow, and 2 calves condemned at an abattoir.....	Aug. 10 Aug. 12	60	1,572,000,000	0.00006	+
	Aug. 13	Tuberculous lesions from 11 guinea pigs inoculated with bovine lesions....		30	107,000,000	0.00001	+

* This represents the weight of tuberculous tissue after grinding but before straining through several thicknesses of cheese cloth. The actual amount of original tissue in each dose is less than one-third of this weight.

† Made by suspending 10 or 20 grams of thoroughly ground and mixed tuberculous tissue in a measured quantity of physiological sodium-chloride solution; then estimating according to the technique for the direct microscopic counting of bacteria in milk.⁴ The estimate was only comparative. The number of organisms consumed was far greater than indicated in this column, since the character of the tissue and the method of estimating did not lend themselves to more definite determination of number of organisms.

‡ The inoculum for the guinea pig was a portion of the ground and strained tuberculous materials suspended in milk as fed to the calves and the weight is given in terms of ground but unstrained material.

§ In the feedings indicated by this mark (\$), acid-fast bacilli were abundant, but no attempt was made to estimate the number of bacilli or the milligrams of tissue injected into control guinea pigs.

and corrals at Berkeley or to a farm at Davis where no other cattle had been kept for several years.

Calmette⁽¹²⁾ has stated that large, frequently-repeated doses of tubercle bacilli given by the mouth will produce pronounced lesions in calves, but, if only one dose is given and the animal is then protected from infection for a time, only slight lesions are produced which render the animal resistant to further infection.

In order to avoid as far as possible this supposed immunizing effect induced by feeding small doses of tubercle bacilli at long intervals, the feeding experiments at the California station have been with large doses at frequent intervals.

An attempt was also made to determine if the prolonged feeding of large doses would produce more extensive lesions than a few feedings. The significance of this attempt will be evident by comparing the graphic symbols for tuberculous lesions in the nonvaccinated control animals listed in table 6. Controls 107-110 received 7 infecting feedings, the dates being September 2, 6, 27, and 29, October 25, and November 27 and 29, while controls *C* and *D* received only the feedings on the last 2 dates. The results in these 2 calves alone are

TABLE 6
RESULTS FROM FEEDING* TUBERCULOUS TISSUE TO CALVES VACCINATED IN THE DEWLAP WITH 50 MG BOCG AND TO CONTROL CALVES

No.	Born	Birth to vaccination	Intervals in days	Tuberculous lesions†						Remarks			
				Vaccination to first feeding	First infecting feeding to autopsy	Number of infecting tubercles	General condition on slaughter	Cervical	Breastfeet	Mesenteric	Medastitis	Lungs	Cecal and coils
7	Apr. 23, 1926	3	129	123	7	Good	7	Good	/	/			No emaciation. One mesenteric lymph node hemorrhagic and guinea pigs inoculated from it became tuberculous. No dewlap lesion.
8	Apr. 26, 1926	4	108	123	7	Good	7	Good	/	/			No lesions except abscess in dewlap, 70 x 30 mm.
9	May 7, 1926	1	127	154	7	Good	7	Good	/	/			Caucous nodule, 4 x 4 mm. in mesenteric lymph node. Guinea pigs inoculated, developed tuberculosis. Connective tissue thickening in dewlap, 20 x 5 mm.
10	May 18, 1926	3	115	154	7	Good	7	Good	/	X			Four 2-mm nodules in retropharyngeal. Many eustocaelearous mesenteric lymph nodes. Guinea pigs became tuberculous. No dewlap lesion.
107	Feb. 24, 1926	Control		123	7	Good	X						Caucosaelearous retropharyngeal 110 mm. Submaxillary 40 mm. Acid-fast bacilli numerous.
108	Mar. 8, 1926	Control		123	7	Poor			X	X			Many caucosaelearous lesions in mesenteric and iliocecal nodes. Acid-fast bacilli numerous.
109	Mar. 13, 1926	Control		154	7	Good	X			X			Retropharyngels enlarged 3 x and caucosaelearous. Ileocecal nodules enlarged 3x and caucosaelearous. Slight mesenteric lesions.
110	Apr. 19, 1926	Control		154	7	Good			/				Gastric node 50 mm and 2 mesenteries caseocalcareous. 2 mesenteries hemorrhagic. Guinea pigs became tuberculous. Postpharyngel had a 35-mm caseous mass. Acid-fast bacilli present.
C	Sept. 30, 1926	Control		36	2	Good	/						
D	Aug. 22, 1926	Control		47	2	Fair	X		/		X		Cervical, mediastinal, and mesenteric caseocalcarous. One 4-mm nodule in lung.
E	July 31, 1926	\$	28	36	2	Poor	X	X					Tuberculous pneumonia evidently from inhalation of infecting feed.
F	Apr. 22, 1926	\$	28	47	2	Poor	X	/		X	X		Entire mesenteric chain caucosaelearous. Cervical and cecal caucosaelearous.

* The animals were given this dose with the aid of a long-necked bottle.

† The symbols for lesions are adopted by federal meat inspectors and the names of the various groups and individual lymph glands correspond in the main to those given by Buckley and Castor.⁶ The adjectives bronchial, mesenteric, etc., referring to lymph nodes are, for brevity, used without the words "lymph nodes". Definitions for slight, well marked, and extensive have been formulated by the writers as a general guide as follows:

/ Slight.—Caucous or caucosaelearous isolated areas less than 15 mm. in diameter.
Well marked.—A single caucous or caucosaelearous area, 15 to 50 mm in diameter. Cases having more than 1 lesion over 25 mm are recorded as extensive.

Extensive.—All progressive or multiple milky tubercles, any one lesion over 50 mm in diameter or two or more lesions over 25 mm.

§ Nos. E and F were vaccinated in the dewlap with 100 mg of bacilli from a culture of acid-alcohol-fast bacilli from a case of bovine lymphangitis (see Cornell Veterinarian 13: 240-246, 1929). These cases are included with their controls, C and D, as an interesting comparison. They received only the last of the 7 infecting feedings given the first 8 calves.

¶ For dates of the infecting feedings see table 6.

inconclusive but are significant when added to the data from the 34 control calves in tables 7B, 8B, and 9B.

Discussion of the Results of Preliminary Infecting Feedings, as Given in Table 6.—The data in this table may be summarized by stating that 7 feedings produced lesions as follows:

1. In 4 calves vaccinated with BCG, 1 extensive, 2 slight, and 1 no-lesion case.

2. In 4 nonvaccinated calves, 4 extensive-lesion cases.

In addition, 3 of the 4 control calves (*C, D, E*, and *F*) that received only the 2 last feedings on November 27 and 29 showed very extensive lesions. The fourth showed well-marked lesions in the cervicals.

The difference between the lesions in the vaccinated and the control calves listed in table 6 was not distinct enough in the writers' opinion to justify a definite conclusion regarding the existence of a protective effect from the BCG vaccination. If present, it evidently was not sufficient to prevent the penetration of virulent bacilli into the cervical or mesenteric lymph nodes and the production of caseous lesions in 2 out of 4 of the vaccinated calves. However, it was decided that further trials by this method of feeding infection might yield more definite information regarding any resistance to tuberculosis which might result from the use of BCG given subcutaneously. Accordingly feeding infection trials were carried out as indicated in tables 7-9.

Discussion of the Results of Ten Infecting Feedings, as Given in Tables 7A and 7B.—From an inspection of table 7A, it will be seen that all the calves which were infected within 25 days after the first vaccination showed well-marked or widely distributed lesions, or both, while in all the other vaccinated calves the lesions were either absent or limited to the cervical and mesenteric or colic regions. Therefore, for purposes of brevity in discussion, the calves in table 7A will be grouped as follows:

1. Calves 11-24 were aged 41 to 407 days at time of first feeding infection, which was given 35 to 320 days after the first vaccination. Of the 14 animals in this group, 5 were found to be apparently normal throughout, 6 showed slight, and 3 showed well-marked caseation or caseocalcification of certain lymph nodes draining the intestinal or pharyngeal regions. All of the carcasses would have been considered as suitable for food under federal meat-inspection regulation. Calf No. 25 died of intercurrent disease too soon after infection to justify consideration.

2. Calves 26-29 were aged 4 to 33 days at time of first feeding infection, which was given from 2 days before to 24 days after vaccination. Three of these had well-marked and widely distributed

TABLE 7A
RESULTS OF TEN FEEDINGS* OF TUBERCULOUS TISSUE TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG
(For controls, see table 7B)
Feeding dates: August 13, 15, 17, 19, 22, 23, 27, September 2, 8, and 9 (For feeding details see table 5)

No.	Born	Birth to vaccination†	Vaccination to first infecting feeding	First infecting feeding to autopsy	Intervals in days	Tuberculous lesions†					Remarks	
						General condition on slaughter	Cervical	Bronchial	Mesenteric and colic	Peritoneal	Medastinal	
11	July 2, 1926	87 (225)‡	320 (182)‡	137	Good							No lesions of tuberculosis except 3 abscesses in dewlap, 40 to 100 mm.
12	Mar. 16, 1927	4	148	338	Good							No lesions of tuberculosis except connective tissue and caseation in dewlap, 40 x 7 mm. Chronic gastritis found from wire.
13	Mar. 18, 1927	2	148	338	Good							No lesions of tuberculosis found. Guinea pigs died of intercurrent disease.
14	Apr. 12, 1927	8	129	332	Fair		/					6 caseous nodules, 2 to 5 mm. in mesenteric and cervical nodes. No dewlap lesion.
15	Apr. 2, 1927	5 (136)	129 (-2)	186	Good		/					9 caseous nodules, 1 to 2 mm., scattered through the mesenteric chain. Guinea pig positive from apparently normal retropharyngeal. Dewlap induration, 60 x 40 x 35 mm.
16	Apr. 12, 1927	5 (101)	142 (46)	323	Good		/					2 nodules, each 2 mm., in mesenteric nodes. Connective tissue mass in dewlap, 60 x 50 x 5 mm.
17	Apr. 24, 1927	6	105	339	Good	\	/					23 caseous nodules, 2 to 8 mm., scattered in mesenteric chain and 1 caseous lesion in retropharyngeal, 5 x 5 x 40 mm.
18	May 18, 1927	8 (41)	79 (46)	360	Good	/						Retropharyngeal, 3-mm caseous nodule. 10 fresh caseous nodules, 1 to 6 mm., scattered in mesenteric nodes. No dewlap lesion.
19	May 26, 1927	9	70	186	Good		/					40 caseous or caseocalcareous nodules, 1 to 10 mm., scattered through the mesenteric chain, and also 1 mass of caseocalcareous nodules, 40 x 20 x 20 mm. Dewlap abscess, 110 mm.
20	June 1, 1927	4 (78)	70 (-2)	351	Good	/						5 post pharyngeal, caseous nodules, 4 to 10 mm. 11 caseous or caseocalcareous nodules, 5 to 20 mm. in mesenteric and cervical nodes. No dewlap lesion.
21	June 11, 1927	9	54	396	Fair							No lesions of tuberculosis except connective tissue thickening in dewlap, 40 x 40 x 20 mm. Tubercles in guinea pigs from apparently normal mesenteric lymph nodes.

TABLE 7B
RESULTS OF TEN FEEDINGS* OF TUBERCULOUS TISSUE IN CONTROL CALVES NOT VACCINATED
Feeding dates the same as in table 7A

No.	Born	Intervals in days	Tuberculous lesions†						Guinea-pig tests	Remarks
			Birth to first infecting feeding	First infecting feeding to autopsy	Cervical	Bronchial	Mediastinal	Lung	Mesenteric	
111	Dec. 29, 1926	227		137						+
112	Jan. 26, 1927	199		338						See remarks
113	Feb. 5, 1927	190		338						
114	Mar. 16, 1927	152		332	/					
115	Apr. 1, 1927	134		186	/					
116	Apr. 13, 1927	117		323		/				
117	Apr. 16, 1927	116		339						
118	May 1, 1927	104		360						
119	May 29, 1927	76		186	×	×	✓			
120	May 31, 1927	74		360		/				
121	June 14, 1927	60		396	/					

* 2 ileocecal nodes enlarged 2 x and caseoceleareous.
 Extensive tuberculosiis of right lung and well-marked lesions in the left lung, caseous foci in mediastinal. Guinea pigs died of intercurrent causes.

† No visible lesions of tubercolosis. The tissue from an apparently normal mesenteric lymph node produced tuberculosis in a guinea pig.

‡ 2 caseous points in a retropharyngeal, 2 and 8 mm, respectively.
 5 caseous nodules in mesenteric chain, 3 to 20 mm in size.
 § Left retropharyngeal one-third caseoceleareous. Mesenteric chain had caseous nodules, 7 to 20 mm, and 1 colic node, caseoceleareous, 18 x 15 mm.

¶ 14 caseous nodules in mesenteric chain, 2 to 12 mm in size.
 3 caseoceleareous nodules, from 4 to 7 mm, in ileocecal lymph nodes.
 Casesons and caseoceleareous lesions, 2 to 25 mm, scattered through the mesenteric and ileocecal lymph nodes.

|| Well-marked caseation in right lung, extensive caseation of retropharyngeal, and thoracic lymph nodes and casesons or caseoceleareous mesenteric lesions.

** 1, 10-mm caseoceleareous nodule in bronchial, 1, 3-mm in post mediastinal and extensive caseocarcification of mesenteric and ileocecal nodes.

*** 3, to 4-mm caseoceleareous nodules in retropharyngeals and extensive caseous and slightly caseoceleareous mesenteric lesions.

TABLE 7A—(Concluded)

No.	Born	Birth to vaccination†	Vaccination to first infecting feeding or autopsy	Intervals in days		Tuberculous lesions‡					Remarks	
				General condition	Infection	Cervical	Breast	Mesenteric	Gastric	Colic		
22	June 13, 1927	7	64	Fair	/	Few	+
23	June 14, 1927	7	53	Good	/	None	—
24	July 3, 1927	6	35	Good	/	Few	+
25	July 14, 1927	6	24	See remarks	None	—
26	July 11, 1927	9	24	137	Good	/	✓	✓	Few	+
27	July 30, 1927	6	8	382	Fair	✓	✓	Few	+
28	July 30, 1927	6	8	380	Fair	✓	✓	Few	+
29	Aug. 9, 1927	6	—2	380	Fair	/	/	/	Few	+

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves except animal No. 11, in which case the dose was administered in a long-necked bottle.

† Tuberculous lesion symbols: / slight, \ well marked, X extensive. For full explanation see footnote table 6.

‡ Figures in parentheses indicate the days to or from the second vaccination.

§ Does not include smears from the dewlap lesions.

¶ Mesenteric, 14, 2 to 10-mm nodules.

Mediastinal, 3, 3-mm nodules.

Dewlap contained white connective tissue thickening, 10 x 5 x 20 mm.

II eusecoelarceous nodules, 2 to 4 mm, scattered in mesenteric chain, one 2-mm nodule in retro-pharyngeal and a nodular connective tissue thickening in dewlap, 40 x 30 x 10 mm.

No lesions of tuberculosis. The dewlap contained a 50-mm thin-walled abscess of soft, creamy, odorless pus. No acid-fast bacilli found.

15 eusecoelarceous nodules, 3 to 12 mm, scattered through the mesenteric and cecal nodes.

No dewlap lesions.

Died of acute gastritis too soon after infection to justify consideration. No tuberculous lesions found.

Each retropharyngeal had a 5-mm necrotic area.

Bronchials had 0.5 to 5-mm caseous areas.

2 mediastinals had well-marked tuberculoses

4, 4 to 10-mm mesenteric lesions. A 17-mm cold

abscess in the dewlap.

Right postpharyngeal enlarged 2X and one-fifth

eusecoelarceous. 9 eusecoelarceous nodules, 4 to

15 mm, scattered through the mesenteric and colic

nodes. Dewlap lesion, 20 mm.

Retropharyngeal cessation 18 x 15 x 12 mm. Slight

lesions in liver and gastrohepatitis. 14 eusecoelarceous nodules, 2 to 12 mm, in mesenteric and

cecal nodes. 5 hard, pea-sized nodules in dewlap.

Vaccinated 2 days after first infecting feeding.

Postpharyngeal, 1, 6-mm nodule. Left bronchial,

8, 3 to 5-mm nodules. Mediastinal, 3, 3-mm

nodules. Mesenteric, 14, 2 to 10-mm nodules.

Dewlap contained white connective tissue thickening, 10 x 5 x 20 mm.

TABLE 7B—(Concluded)

No.	Born	Intervals in days Birth to first infecting feeding	First infecting feeding to autopsy	Tuberculous lesions†						Acid- fast bacilli found in smears	Guinea- pig tests	Remarks
				Cervical	Medastinal	Pronothal	Lungs	Mesenteric	Gastric and colic and colic lesions			
122	June 15, 1927	59	396	/	X	/	/	/	/	Many	+	2 caseocalcareous nodules, 4 to 5 mm in mediastinal. Extensive caseocalcareous lesions in the mesenteric and cecal lymph nodes. Caseocalcareous areas, 20 and 10 mm, in left postpharyngeal, 12 mm in right postpharyngeal, 4 mm in mediastinal, and 7 mm in a colic lymph node.
123	June 22, 1927	52	380	/	X	/	/	/	/	Few	+	Caseocalcareous areas, 20 and 10 mm, in left postpharyngeal, 12 mm in right postpharyngeal, 4 mm in mediastinal, and 7 mm in a colic lymph node.
124	July 5, 1927	39	360	X	/	X	X	/	/	Few	+	A soft caseous lesion, 60 mm, in left postpharyngeal and 1, 10-mm in gastrohepatic. Extensive caseation in mesenteric and well-marked caseocarcinomatous lesions in ileo-cecal.
125	July 14, 1927	30	37				X			Many	+	Killed 37 days after infection because of the death of vaccinated calf No. 25, of which it was the control. Mesenteric lymph node was necrotic, 30 mm.
126	July 22, 1927	22	137	X	/	/	/	/	/	Few	+	Retropharyngeal enlarged 2 X, caseous throughout. 8 caseocarcinomatous areas, 1 to 3 mm, in mesenteric and cecal nodes.
127	July 23, 1927	21	382	/	X	/	X	/	/	Many	+	Lungs studded with caseocarcinomatous nodules. Caseous lesions in mediastinal and caseocarcinomatous lesions in retropharyngeal, bronchial, and pulmonary lymph nodes.
128	Aug. 6, 1927	7	389							Many	+	Only 1 caseocarcinomatous 7-mm nodule in center of mesenteric chain of lymph nodes.
129	Aug. 7, 1927	6	380	/						Many	+	Liver studded with 3 to 10-mm caseous nodules. Caseous lesions in mesenteric and mediastinal nodes and caseocarcinomatous in pharyngeal and gastrohepatic.

* The infecting dose as described on table 5 was placed in a bucket and was voluntarily consumed by the calves, except animal No. 111, in which case the dose was administered in long-necked bottles.

† Tuberculous lesion symbols: / slight, \ well marked, X extensive. For full explanation see footnote, table 6.

‡ No smears made.

TABLE 8A
RESULTS FROM THIRTY FEEDINGS* OF TUBERCULOUS TISSUES TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG
(For controls, see table 8B)

Feeding dates: August 13 to September 9 as in tables 7A and 7B and also September 10, 12, 14, 16, 18, 19, 24, 28, 29, 30, and October 1-8, 11, and 12, 1927

No.	Born (1927)	Intervals in days		Tuberculous lesions†				Acid-fast bacilli found in smear§	Remarks
		Birth to vaccina- tion	Vaccina- tion to first feeding injecting autopsy	Cervical	Mesenteric	Gastric	and colic		
30	Mar. 30	8 (91)§	120 (49)§	186	Good	/	/	Few	+
31	Apr. 4	5	124	323	Good	/	/	Few	+
32	Apr. 13	9 (76)	113 (46)	331	Fair	/	/	Few	+
33	Apr. 26	4	105	380	Good	/	/	None	+
34	May 5	8	79	323	Good	/	/	None	-
35	May 30	6	70	339	Good	/	/	Few	+
36	June 1	3 (27)	70 (46)	382	Good	/	/	None	+
37	June 5	4	64	389	Fair	/	/	None	+
38	June 10	10	54	192	Good	/	/	Few	+
39	June 14	6	54	331	Poor	X	/	Few	+

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.

† Tuberculous lesion symbols: / slight, \ well marked, X extensive. For further description see footnote, table 6.

‡ Does not include smears from the dewlap lesions.

§ Figures in parentheses indicate the days to or from second vaccination.

Caseation in 7 mesenteric nodes, 2 to 18 mm. Dewlap had 60-mm con-
nective tissue thickening.
2 calcareous 5-mm nodules in mesenteric lymph nodes. Dewlap abscess,
40 mm, contained acid-fast bacilli. (See fig. 11.)
Caseous nodule in spleen, 18 x 12 x 7 mm. Guinea pigs tuberculous from
this spleen lesion. 32 caseocalcareous nodules, 2 to 7 mm, scattered in
mesenteric and cecal nodes.
12, 2 to 5-mm caseocalcareous nodules in mesenteric nodes, and 4, 2 to
4-mm in cecal nodes. Dewlap had slight thickening of firm white con-
nective tissue.
No lesions except 50-mm cold abscess in dewlap, containing yellow, soft,
odorless pus and a few acid-fast bacilli.
Soft caseous lesions in both retropharyngeal, 12 caseous areas in ileocecal
nodes and 16 in mesenteric chain, ranging from 2 to 20 mm. No dewlap
lesion.
Right retropharyngeal and 1 gastrohepatic each had 1, 4-mm caseous
nodule. 31 calcareocaseous areas, 5 to 10 mm, in mesenteric or cecal nodes.
Abscess in dewlap, 40 mm, contained acid-fast bacilli.
1, 4-mm caseous nodule in right retropharyngeal and 15 caseocalcareous
nodules, 3 to 8 mm, in mesenteric chain. Dewlap small abscess.
13 caseocalcareous nodules, 1 to 10 mm, in mesenteric chain and 3, 1-mm
caseous points in an ileocecal node. Small caseous lesions in dewlap
contained acid-fast bacilli. (See figs. 11 and 12.)
20 soft caseous areas, 7 to 30 mm, in mesenteric chain. No dewlap abscess.

TABLE 8B
RESULTS FROM THIRTY FEEDINGS* OF TUBERCULOUS TISSUE IN CONTROL CALVES NOT VACCINATED
Feeding dates: August 13 to October 12, 1927, as in table 8A

No.	Born (1927)	Interval in days	Birth to first infecting feeding	First infecting feeding to autopsy	Tuberculous lesions†						Acid-fast bacilli in smears	Guinea-pig tests	Remarks
					Cervical	Bronchial	Medastinal	Mediastinal	Cecal	Gastric			
130	Apr. 1	107		186	/	/	Many	+	About one-half the mesenteric and ileocecal lymph nodes were caseocelecarous. Right bronchial and right prescapular nodes each had a small caseous lesion. 12 caseocelecarous and 5 calcareous nodules, 3 to 20 mm in size, scattered through the mesenteric chain.
131	Apr. 14	121		323	Many	+	
132	Apr. 16	119		331	/	Many	+	
133	Apr. 25	110		380	Many	+	
134	May 26	79		323	Many	+	
135	May 31	74		339	/	Many	+	
136	May 31	74		382	Many	+	
137	June 7	67		389	Few	+	
138	June 8	66		192	Many	+	
139	June 12	62		331	Many	+	

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.

† Tuberculous lesion symbols: / slight, \ well marked, X extensive; for further details see footnote, table 8.

TABLE 9A

RESULTS FROM THIRTY-SIX FEEDINGS* OF TUBERCULOUS TISSUE TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG

(For controls, see table 9B)

Feeding dates: * August 13 to October 12, 1927, as in tables 8A and 8B, and also October 14, 15, 16, 17, 19, and 22, 1927

No.	Born	Intervals in days	Tuberculous lesions*						Acid-fast bacilli found in smear†	Guinea-pig tests	Remarks
			General condition on slaughter	Cervical	Bronchial	Mesenteric	Lungs	Mediastinal			
40	May 16	2 (27)†	451 (182)†	132	Good	/	/	/	None	+	
41	June 20	8 (56)	46 (-2)	397	Poor	/	/	/	+	+	
42	July 4	5 (42)	35 (-2)	140	Good	/	/	/	+	+	
43	July 12	8	24	383	Good	/	/	/	None	+	
44	Aug. 6	9	-1	215	Poor	/	/	/	+	+	

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.

† Figures in parentheses indicate the days to or from second vaccination.

‡ This column does not include smears from dewlap lesions.

§ Tuberculous lesion symbols: / slight, \ well marked, X extensive. For further description see footnote, table 6.

TABLE 9B
RESULTS FROM THIRTY-SIX FEEDINGS* OF TUBERCULOUS TISSUE TO CONTROL CALVES, NOT VACCINATED
Feeding dates: August 13 to October 12, 1927, as in tables 8A and 8B, and also October 14, 15, 16, 17, 19, and 22, 1927

No.	Born	Interval in days	Tuberculous lesions†						Remarks
			Birth to first infecting feeding	First infecting feeding to autopsy	General condition on slaughter	Cervical	Bronchial	Mesenteric	
140	1926 Oct. 14	303	132	Good	X				+
141	1927 June 13	61	397	Poor	/ /	/ \	X	/	+
142	July 13	31	140	Fair	X	/		/	+
143	Aug. 5	8	383	Fair				/ /	+
144	Aug. 7	6	215	Fair	\			/ /	+

*The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.
† Tuberculous lesion symbols: / slight, \ well marked, X extensive. For further description see footnote, table 6.

caseous or caseocalcareous lesions and the other calf had slight, but widely distributed lymph-node lesions. A literal and strict interpretation of the federal meat-inspection regulations would have resulted in the condemnation of all 4 carcasses.

Calves 11 to 24 differed from Nos. 26 to 29 in two respects, i.e., in the length of time after vaccination that they were infected and in their ages, the latter group being younger. A comparison of tables 7A and 7B shows that age was probably not a significant factor since well-marked or extensive lesions occurred in the nonvaccinated calves infected at ages from 6 to 199 days. The comparison also shows that, in general, the tuberculous lesions were more extensive in the control than in the vaccinated calves. It would seem, therefore, that the subcutaneous injection of BCG resulted, after 4 or 5 weeks, in the development of a certain resistance to tuberculosis. However, it was definitely shown that this resistance was not usually sufficient to prevent the penetration of the tissues of the alimentary tract by virulent tubercle bacilli and the production of caseous lesions in the mesenteric or cervical lymph nodes.

Discussion of the Results of Thirty Infecting Feedings, as Given in Tables 8A and 8B.—It will be noted from the table 8A that 9 of the 10 vaccinated calves had caseous or caseocalcareous lesions. In all but 1 of these, the visible tissue involvement was confined to the lymph nodes draining the pharyngeal or intestinal tracts. The exception was No. 32, in which was found a single tuberculous caseous nodule in the spleen, as well as 32 small caseocalcareous areas scattered through the lymph nodes draining the intestines. All 10 of the controls had well-marked or extensive caseous or caseocalcareous tuberculous lesions in 2 or more groups of lymph nodes.

On comparing the lesions in the vaccinated calves with their respective controls killed on the same date, it was observed that in one instance the lesions in the vaccinated calf (No. 36) were more widely distributed than in its control (No. 136) but not so extensive. The tissue involvement in calves 32 and 35 was about equal to that involved in their controls, 132 and 135. In the other 7 vaccinated calves, the lesions involved less tissue than in their respective controls. The results in tables 8A and 8B support the observations outlined in tables 7A and 7B, in that it is definitely shown that the vaccination with BCG did not prevent the penetration of the tissues of the alimentary tract by virulent tubercle bacilli and the production of caseous and caseocalcareous lesions in the adjacent lymph nodes. In general, however, the tuberculous lesions were more extensive in the control than in the vaccinated calves.

Discussion of the Results of Thirty-six Infecting Feedings, as Given in Tables 9A and 9B.—The 10 animals in this group had received 30 infecting feedings with the 20 calves listed in tables 8A and 8B, and it was decided to note what would result from the feeding of 6 more very massive doses of bovine tuberculosis lesions suspended in milk. On completing the autopsies, it was noted that, in general, no very marked differences existed, either in the extent or distribution of the lesions, which could be attributed to the additional infecting feedings.

All of the vaccinated calves showed widely distributed caseous or caseocalcareous tuberculous lesions in the lymph nodes. These lesions were small, ranging from a pin point to 10 mm. One retropharyngeal lesion, 35 mm in diameter, was found in calf 41, and small tuberculous lesions were also demonstrated in Peyer's patches of calf 42.

All of the control calves had widely distributed well-marked or extensive caseous or caseocalcareous tuberculous lesions of the lymph nodes. Pulmonary and hepatic tuberculosis existed in calf 141 and Peyer's patch tuberculosis in calf 142.

In general, the extent of the lesions in the vaccinated animals was less than in the controls and these findings substantiated the similar observations in the previous infection trials at this Station.

A comparison of the lesions in the calves which were revaccinated at about the beginning of the infecting feeding (Nos. 15, 20, 41, and 42) with the autopsy findings in other vaccinated calves revealed no significant difference. Therefore it would appear that the subcutaneous revaccination of calves during a time when they are exposed to infection will probably not result in any appreciable difference in their lesions.

It should be noted that calf 44, at 9 days of age, was vaccinated for the first and only time on the day after starting the infecting feedings. This calf was unthrifty and was slaughtered when 224 days of age or 215 days after the first infecting feeding. The animal was emaciated and its musculature was edematous. In spite of this, the only tuberculous lesions found were slight caseations of the cervical and abdominal lymph nodes. A guinea pig inoculated from portions of these lesions developed tuberculosis. Another guinea pig inoculated from portions of other tissues remained normal.

Discussion of the Results of Feeding Milk from Tuberculous Udders, as Given in Tables 10A and 10B.—The 4 calves, Nos. 45, 46, 145, and 146, received feedings daily for 63 days from the tuberculous udder of cow No. 600 (see fig. 9). This cow was milked twice daily,

TABLE 10A
RESULTS FROM FEEDING* MILK FROM TUBERCULOUS UDDERS TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG

No.	Born (1927)	Intervals in days		Tuberculous lesions†					Guinea-pig inoculation	Remarks
		Birth to vaccination	Vaccination to infection	General condition on slaughter	Cervical	Bronchial	Medastinal	Mesenteric	Lunges	
45	Sept. 11	6	77	Fair					/	+
46	Sept. 14	3	77	Good					/	-
47	Sept. 12	5	13	94	Fair	/	/	/	Many	+
48	Sept. 13	4	13	246	Died, see remarks	/				Few

* The feedings were either by direct nursing of cow, or else by pail feeding.

† Tuberculous lesion symbols: / slight, \ well marked, X extensive. For further description see footnote, table 6.

‡ Does not include smears from the dewlap lesions.

Protected from tuberculous infection until the 83rd day of life. Then fed for 63 days on milk from cow 600 (see page 352). Butchered at 186 days of age. A lymph node of the mesenteric chain contained 6 caseous foci, pin point to 1 mm in size and a caecal node contained a 20-mm caseous mass. The dewlap connective tissue was thickened 20 mm and contained acid-fast bacilli.

Protected from tuberculous infection until the 80th day of life. Then fed on milk from cow 600 (see page 352) for 63 days. Butchered at 307 days of age. No lesions resembling tuberculosis were found. Guinea pigs injected with cervical, thoracic, and abdominal lymph nodes remained normal. The dewlap contained a 70-mm thickening, but no acid-fast bacilli were found in it.

Born and reared until the 18th day of life in a tuberculosis-free herd. Then fed daily for 86 days, Sept. 30 to Dec. 25, 1927, on the milk from cow 2171 (see page 352). Butchered at 112 days of age, 4, 2 to 8-mm necrotic foci in retropharyngeals. One-third the volume of the bronchial and mediastinal nodes was caseous, 2, 2 to 5-mm nodules in the lung. All the caecal nodes were enlarged and caseous. Other portions of the mesenteric chain had 3 or 4, 1 to 2-mm necrotic points in many lymph nodes. A dewlap abscess, 37 mm, contained acid-fast bacilli.

Same age, history, and period of infection as calf 47. Found dead in field at 263 days of age. Cause of death not known. Fair general condition of carcass. Left retropharyngeal had 9, 2-mm millet-like nodules grouped in a volume of less than 1 cc and the right retropharyngeal contained 13 such nodules in a group measuring about 1 cc. A 40-mm cyst in the dewlap contained acid-fast bacilli.

TABLE 10B
RESULTS FROM FEEDING* MILK FROM TUBERCULOUS UDDERS TO CONTROL CALVES NOT VACCINATED

No.	Born (1927)	Purpose	Birth to infection	Intervals in days	Tuberculous lesions†						Acid-fast bacilli found in smears	Guinea- pig inoculation	Remarks
					General condi- tion on slaugh- ter	Cervical	Bronchial	Mediastinal	Other	Liver			
145	Sept. 14	Control on 45 and 46	80	103	Fair	/	/	/	/	/	Many	+	Protected from infection to 80th day of life. Then fed for 63 days on milk from cow 600. Butchered at 183 days of age. Left retropharyngeal studded with caseous masses. Posterior mediastinal had a 4-mm caseous focus. One mesenteric had a 4-mm caseous focus.
146	Sept. 16	Control on 45 and 46	78	227	Good	/	/	/	/	/	Many	+	Protected from infection to 78th day of life. Then fed for 63 days on milk from cow 600. Butchered at 365 days of age. Parotid lymph node had a 4-mm caseous focus. In the mesenteric lymph nodes were caseocelecarious masses as follows: 3, 4-mm; 1, 10-mm; 1, 25-mm, and in the ileocecal 1, 25-mm.
147	Sept. 12	Control on 47 and 48	18	62	Died, see re- marks	×	×	×	×	×	Many	+	Protected from infection to 18th day of life. Then fed until death (62 days) on milk from cow 2171. Died of tuberculosis at 80 days of age. Generalized pulmonary and lymphatic tuberculosis.
148	Sept. 14	Control on 47 and 48	16	162	Died, see re- marks	×	×	×	×	...	Many	+	Protected from infection to 17th day of life. Then fed for 86 days on milk from cow 2171. Died of tuberculosis at 176 days of age. Generalized pulmonary and lymphatic tuberculosis. Lesions were caseocelecarious.

* The feedings were either by direct nursing of cow, or else by nail feeding.

† Tuberculous lesion symbols: /, slight, \, well marked, X, extensive. For further description see footnote, table 6.

and the mixed secretion from all 4 teats was given to the 4 calves. An effort was made to give each calf approximately the same amount of milk. Acid-fast bacilli were demonstrated in smears from the left front quarter of the udder of cow No. 600 on several occasions, and, on autopsy, the parenchyma of the right rear and left front quarters was found to contain massive tuberculous lesions. This cow also had extensive pulmonary tuberculosis, and the calves were confined in an adjacent pen where she could reach over and lick them. In spite of the obviously massive infection to which the 4 calves were thus exposed, none of them showed lesions on autopsy which were extensive according to the classification used in these trials. In one of the vaccinated calves (No. 46), no lesions were found, and guinea pigs inoculated with bits of tissue from various lymph nodes remained normal. The other vaccinated calf had well-marked caseation of certain lymph nodes draining the intestine, as indicated in the table. The lesions in the 2 controls were more pronounced than in the vaccinated calf which showed lesions.

Cow No. 2171 (see fig. 10), from whose udder infecting feedings were given to calves Nos. 47, 48, 147, and 148, had been artificially infected on August 2 by thrusting a needle 2 inches into the glandular tissue of the right front and rear quarters and injecting spleen and lymph-node pulp from a guinea pig infected with bovine tuberculous lesions. As a result, the milk of these quarters was found to be virulent for guinea pigs on September 8 and 29, October 19, and November 5. Acid-fast bacilli were seen in smears of the milk of the 2 right quarters at various times between September 8 and November 25, 1927. On autopsy of cow No. 2171 on November 30, 1927, a tuberculous, necrotic focus was found in the parenchyma of each of the right quarters. Each focus was about 10 cm in diameter. No other lesions of tuberculosis were found in this cow.⁹

Calf No. 48 died in the pasture from unknown causes and was not found for 3 days. Post-mortem changes rendered the autopsy difficult; however, it is believed that no well-marked lesions of tuberculosis were present. Guinea pigs, inoculated from a postpharyngeal lesion in this calf, became tuberculous.

It is noteworthy that calves 47 and 48 were subjected to the infecting feedings only 13 days after vaccination when it might be supposed that the full immunizing effect of the vaccination had not yet developed.

⁹ Such limited localization of lesions resulting from inoculation of tuberculous material into the udder is unusual according to Cobbett. (26)

The control calves, were of about the same age and size as the vaccinated. They were reared and fed together and were comparable in every way except for the vaccination in the dewlap.

It will be noted that an evident difference exists in the susceptibility of the vaccinated calves listed in table 10A and the unvaccinated calves listed in table 10B. Two out of 4 controls died of tuberculosis, while all of the vaccinated animals remained in good or fair condition up to the time of death.

These findings are of significance when added to the data in previous tables because they substantiate the results already obtained—that the subcutaneous vaccination will not prevent the penetration of the tissues by virulent tubercle bacilli but the lesions remain localized in the lymph nodes draining the points of entry of the bacilli, while in nonvaccinated calves the result may be a generalized tuberculosis.

INTRAVENOUS, ORAL, AND INTRADERMIC VACCINATION

It was decided to test the protective effect, if any, of vaccination of calves by various other methods besides the injection into the dewlap as recommended by Calmette and Guérin.⁽²⁰⁾ Accordingly, as indicated in table 11A, 3 calves were vaccinated intravenously, 6 were given 3 vaccinating feedings each, and 3 were injected intradermally. As in previous trials the vaccinated animals and an equal number of controls were carefully protected from tuberculous infection until the time they were subjected to infection by feeding tuberculous bovine tissues suspended in milk. A few days before the first infecting feeding the controls were given an intradermic tuberculin test, but no reactions occurred. The detailed preparation of the infecting material will be found in table 5, and the results are summarized in tables 11A and 11B.

Discussion of Tables 11A and 11B.—It will be noted that in each group 6 animals received only 1 infecting feeding, while the other 6 received 3 infecting feedings. A study of the controls in table 11B indicates that the 1 feeding on July 7 was as effective in producing extensive lesions as the 3 feedings on July 7, 9, and 11, since extensive lesions occurred in every one of the control calves which received the 1 feeding on July 7, while in 2 of the calves which received 3 feedings, the lesions were only well marked. This supports the data already presented in tables 6 to 9, showing that continued feeding of large doses of virulent tubercle bacilli has little apparent effect in increasing the severity or extent of the lesions produced.

TABLE II
RESULTS FROM FEEDING TUBERCULOUS TISSUES TO CALVES VACCINATED INTRAVENOUSLY, BY MOUTH, OR INTRADERMALLY

No.	Born (1928)	Vaccination with BCG	Number of infected feedings*	Intervals in days	Tuberculous lesions†						Remarks		
					First vaccination to first infection	First injection to autopsy	General condition	Cervical	Bronchial	Mesenteric	Medastinal		
49	Jan. 27	Intravenously: 100 mg. Feb. 3	1	155	207	Good	/	/	/	/	/	None	+
50	Jan. 5	By mouth: Jan. 16, 1 ggm Jan. 18, 1 ggm Jan. 20, 1 ggm	1	173	192	Good	/	/	/	/	/	Few	+
51	Apr. 9	By mouth: Apr. 13, 3 gms Apr. 15, 2 gms Apr. 17, 2 gms	1	85	186	Fair	/	/	/	/	/	Few	+
52	Apr. 10	By mouth: Apr. 13, 3 gms Apr. 15, 2 gms Apr. 17, 2 gms	1	85	186	Fair	/	/	/	/	/	Few	+
53	Jan. 27	Intradermally: 100 mg. Feb. 3	1	155	207	Good	/	/	/	/	/	Few	+
54	Feb. 21	Intradermally: 100 mg. Feb. 29	1	129	3	Died	/	/	/	/	/	None	-

* 2 mesenteric lymph nodes, each having 1, 2-mm caseous point.

† 2, 2-mm caseous foci in retropharyngeal, 5, 1 to 2-mm in pulmonary, 31 caseoecalcarous nodules 1 to 12 mm in mesenteric, and 3, 2 to 3-mm in ileocecal lymph nodes.

Right retropharyngeal lymph node had 15 caseous foci 1 to 4 mm in size.
".

9 caseous points 1 to 4 mm in size in left, and 17 in right retropharyngeal lymph node.

A 2-mm caseous nodule in right retropharyngeal lymph node.

Died of gastroenteritis too soon after infection to justify consideration. No tuberculous lesions were found.

"

TABLE 11B
RESULTS FROM FEEDING* TUBERCULOUS TISSUES TO CONTROL CALVES KEPT WITH THE ANIMALS LISTED IN TABLE 11A

No.	Born (1928)	Num-ber of infect-ing feed-ings	Intervals in days	General condition at autopsy	Tuberculous lesions†						Acid-fast bacilli found in smears	Guinea-pig inoculation	Remarks
					Birth to first infecting feeding	First infecting feeding to autopsy	Cervical	Bronchial	Medastinal	Lungs			
149	Jan. 22	1	167	Fair	X	/	X	X	X	/	Many	+	Extensive caseous lesions in liver and in Peyer's patches of small intestines. Extensive caseous and eosinocarcinous masses in gastrohepatie, mesenteric, and cervical, and slight caseation of bronchial and mediastinal. Well-marked caseation of Peyer's patches and extensive soft caseation of mesenteric and right retropharyngeal, and small caseous foci in mediastinal and cecal lymph nodes.
150	Jan. 21	1	166	207	Good	X	X	X	X	/	Few	+	Lower third of mesenteric chain extensively caseo-carcinous. Massive caseocalcification in retropharyngeal. Scattered cecal and colic node lesions.
151	June 4	1	33	186	Fair	X	X	X	X	/	Many	+	Extensive caseation of retropharyngeals and a few caseo-carcinous foci in submaxillary, mediastinal, mesenteric, and cecal nodes.
152	June 15	1	22	186	Fair	X	X	X	X	/	Many	+	Numerous 2 to 4-mm nodules in liver. Extensive caseation of gastrohepatie; well-marked in the retropharyngeal and mesenteric and slight in the bronchial, mediastinal, pulmonary, and cecal nodes.
153	Jan. 24	1	169	207	Good	\	/	/	/	/	Few	+	Well-marked Peyer's patch tuberculosis and extensive or slight lymphatic caseation as indicated in symbol columns of this table.
154	Feb. 25	1	132	191	Fair	X	/	/	X	/	Many	+	

TABLE 11A—(Concluded)

No.	Born (1928)	Vaccination with BCG	Number of infect- ing feed- ings*	Intervals in days		Tuberculous lesions†				Guinea- pig inocu- lations‡	Remarks
				First vacina- tion to first infec- tion	First infection to autopsy	Cervical	Bronchial	Mesenteric and colic	Other lesions		
55	Jan. 31	100 mg, Feb. 3	3	155	192	Fair	/	Few	+
		By mouth:				Died	/	Few	+
56	Feb. 3	Feb. 8, 1 gm	3	150	160
		Feb. 10, 1 gm			
		Feb. 12, 1 gm			
57	Feb. 26	By mouth:		3	131	207	Good	/	/	+
		Feb. 27, 1 gm			
		Feb. 29, 1 gm			
		Mar. 2, 1 gm			
58	Apr. 13	By mouth:		3	85	186	Fair	None	+
		Apr. 13, 3 gms			
		Apr. 15, 2 gms			
		Apr. 17, 2 gms			
59	Feb. 1	Intradermally:		3	155	191	Fair	/	Few	+
		100 mg, Feb. 3			
60	June 8	Intravenously:		3	53	152	Fair	None	+
		100 mg, June 18			

* The infecting feedings were given through a rubber tube. For details regarding dates and dosage see table 5.

† Tuberculous lesion symbols: / slight, \ well marked, X extensive. For details see footnote, table 6.

‡ Does not include smears from the dewlap lesions.

TABLE 11B—(Concluded)

No.	Born (1928)	Num- ber of infect- ing feed- ings	Intervals in days	Tuberculous lesions†										Guinea- pig inocu- lation	Remarks		
				Birth to first infecting feeding	First infecting feeding to autopsy	Cervical	Bronchial	Mediastinal	Mesenteric	Lungs	Cecal coli	Gastric hepatico- celiac	Gastro- celiac	Liver	Other lesions		
155	Jan. 21	3	166	207	Good	✓	/	/	/	/	/	/	/	/	None	+	Well-marked caseocalcification in retropharyngeal and mesenteric and slight in pulmonary, bronchial, mediastinal, and cecal nodes.
156	Feb. 5	3	152	160	Good	✗	✓	✓	✗	✗	✓	✓	✓	✓	Few	+	Extensive caseocalcification of mesenteric, cecal, colic, retropharyngeal, and a few 2 to 3-mm nodules in bronchial, mediastinal, and gastrohepatic lymph nodes. Well-marked caseation in Peyer's patches.
157	Apr. 14	3	84	191	Fair	✗	✓	✓	✓	✓	✓	✓	Few	+	24, 1 to 12-mm caseoareaceous nodules in mesenteric, 1, 3-mm in cecal, 1-4-mm in a Peyer's patch, and extensive caseocalcification of the retropharyngeal lymph nodes.
158	June 15	3	22	186	Fair	✗	...	✓	...	✓	✓	✓	✓	✓	Many	+	Extensive caseocalcification in mesenteric and ileocecal, and well-marked in gastrohepatic and mediastinal, 2-mm foci in gastrohepatic and mediastinal.
159	Feb. 25	3	132	191	Fair	✓	...	✓	✓	✓	✓	✓	Many	+	Extensive caseocalcification in mesenteric chain. 1 caseous mass in a Peyer's patch, cecal nodes studded with 2 to 3-mm nodules. 1, 1-mm nodule in a mediastinal node.
160	June 5	3	66	152	Fair	✓	✓	✓	✓	✓	✓	None	+	3, 15 to 22-mm caseoareaceous masses in retropharyngeal and 84 in mesenteric, cecal, and colic lymph nodes.

* The infecting feedings were given through a rubber tube. For details regarding dates and dosage see table 5.

† Tuberculous lesion symbols: / slight, \ well-marked, X extensive. For details see footnote 6.

From table 11A it will be noted that the time elapsing between vaccination and infection ranged from 53 to 173 days. The lesions in the vaccinated calves were well marked in 2 cases (Nos. 57 and 59), slight but widely distributed in 1 (No. 50), slight and localized in 6 (Nos. 49, 51, 52, 53, 55, and 56) and in 2 (Nos. 58 and 60) no evidence of tuberculous infection could be found. No. 54 died of intercurrent disease and was not taken into consideration. In contrast, the controls all had well-marked or extensive widely distributed tuberculous lesions.

The number of calves used in the trials described in table 11A is not sufficient to justify final conclusions, but a comparison with the results in the controls (table 11B) supports the evidence obtained in the previous trials of BCG given subcutaneously. A resistance to subsequent infection is produced by the presence of the BCG organisms in the tissues; as a rule, however, this is not sufficient to prevent the penetration of the body by virulent tubercle bacilli and the production of caseation or caseo-calcification in the lymph nodes.

From the limited number of calves used (12), there is nothing to indicate that the administration of the BCG vaccine by mouth, intravenously, or intradermally had any more immunizing effect than when given subcutaneously in the dewlap.

THE USE OF BCG IN A TUBERCULOUS HERD

On the first visit to the Williamson farm on Grisly Island in April, 1926, to begin the use of BCG, it was evident that extensive tuberculous infection existed. Several of the cows had enlarged retropharyngeal or prescapular lymph nodes, and a few were observed with symptoms of advanced pulmonary lesions. Guinea-pig inoculations from 2 of the animals proved the existence of cases of open tuberculosis in the herd. An intradermic tuberculin test, applied to all the cattle in the herd, showed 55 per cent positive reactors. The results of this test, grouped according to the ages of the cattle, are given in table 12.

The calves born on this dairy since 1920 had been permitted to nurse their dams for from 2 to 5 days, after which they were transferred to nurse cows or fed from pails on the mixed raw milk or skim milk of the entire herd.

In 1926 at the time the experiment was started, Calmette and Guérin had not retracted their published statement that it was unnecessary to take special sanitary precautions or to heat the milk from the tuberculous cows before feeding it to the calves. It was evident to

the writers that the vaccine would be more likely to be effective if the calves could be protected from infection for at least 30 days after vaccination. However, the owner desired to proceed with the trial on the basis of the published recommendations of Calmette and Guérin existing at that time, and it was decided to accede to his urgent request in the hope that the majority of the calves would escape infection during the first month of life and that by repeated vaccination over a period of several years, it would be possible to build up a resistant herd.

TABLE 12

TUBERCULIN TEST, H. D. WILLIAMSON DAIRY, APRIL, 1926

One-tenth cc of 10 per cent solution alcohol-precipitated tuberculin was injected into left subcaudal fold.

Age	Number tested	Number positive reactions	Number indecisive reactions	Number negative
Less than one year.....	27	4	0	23
1 to 2 years.....	38	23	0	15
2 to 3 years.....	43	18	0	25
3 to 4 years.....	25	14	1	10
4 to 5 years.....	20	13	0	7
5 to 6 years.....	15	10	0	5
6 to 7 years.....	19	14	2	3
7 to 8 years.....	29	23	2	4
8 to 9 years.....	1	1	0	0
10 to 15 years.....	4	3	0	1
15 to 20 years.....	2	0	0	2
Total.....	223	123	5	95

During 1926, 38 calves were vaccinated and 10 were left unvaccinated as controls. Since that time, few controls have been left, because the owner insisted on having all of the calves vaccinated in the hope that sufficient resistance would be created to prevent the development of any open cases of tuberculosis in the vaccinated animals and that eventually he could dispose of all unvaccinated cattle, cease vaccination and develop a nonreacting herd from the offspring of the vaccinated animals.

The vaccinating dose used in 1926 was 50 mg. Since that year in all cases the dose has been 100 mg injected into the dewlap, the first injection being given from 1 to 10 days after birth. The revaccinations have been made in 4 to 10 months following the initial injection and annually thereafter. The numbers of calves vaccinated each year are listed in table 13.

In accordance with the recommendations of Calmette, no tuberculin has been used on the vaccinated calves since 1926, with the exception

of 4 animals just before slaughter. The owner was advised that, if tested, they would probably react for a time as a result of the vaccination regardless of whether or not they developed tubercles other than those in the dewlap. (See the section "Hypersensitiveness in Vaccinated Calves," pp. 366.) He was also advised in 1927 that the most that could be expected from the vaccine was a resistance and that it would not prevent the penetration of the tissues by virulent tubercle bacilli. However, he decided to continue the vaccination of all the calves.

TABLE 13
CALVES VACCINATED AT H. D. WILLIAMSON DAIRY

Year born	Number vaccinated				
	First time	Second time	Third time	Fourth time	Fifth time
1926.....	38	33	26	25	22
1927.....	58	51	50	43
1927.....	6	6	6
1928.....	44	40
1929.....	46
Total.....	192

The following year he had some reason to hope that the majority of them would escape infection during the first 30 days of life because 3 vaccinated calves, reared on the place for from 8 to 12 weeks, had been taken to Berkeley and no tuberculous lesions were found on slaughter after 9, 16, and 18 months, respectively. On the other hand, a nonvaccinated calf of this group was found to be tuberculous on slaughter.

Guinea-pig inoculations from the mixed milk of the herd on one occasions in 1927 resulted in producing tuberculosis.

In September, 1927, one of the 6-week-old calves which had been vaccinated 5 days after birth was observed with labored breathing and coughing. On slaughter at 2 months of age, extensive tuberculous lesions were found in the lungs and thoracic lymph nodes. A guinea pig inoculated from these lesions died of generalized tuberculosis, but a guinea pig inoculated from the vaccination abscess in the dewlap was normal throughout when slaughtered 2 months after inoculation. On investigation, it was found that the cow on which this calf had nursed had tuberculosis of the udder (see cow No. 600, page 352). A non-vaccinated control calf which had been nursing on this cow also developed pulmonary symptoms and on slaughter was found to have tuberculosis of the mesenteric lymph nodes and non-

tuberculous bronchopneumonia. With the previous exception, none of the vaccinated calves in this herd has apparent clinical tuberculosis.

In the winter of 1928, 2 of the nonvaccinated controls, born in 1926, were reported by the owner to have died of tuberculosis. Unfortunately, no tissues were saved and the University veterinarian was not notified.

During 1929 it has been possible to perform autopsies on 10 of the 3-year-old heifers, 5 of which had been repeatedly vaccinated and 5 of which were nonvaccinated controls. The results are given in tables 14A and 14B.

Discussion of Tables 14A and 14B.—The extent of exposure of the cattle in this field experiment to tuberculous infection cannot be as satisfactorily measured as the controlled infection trials at Berkeley and Davis. However, from 8 nonvaccinated animals available for tuberculin testing or slaughter, 5, or 62.5 per cent, developed tuberculosis. These 5 are listed in table 14B. One of them, No. 205, was killed because it was in an extremely poor and weak condition as a result of tuberculous infection. The other 4 were selected from 7 available nonvaccinated animals because they yielded strong positive reactions to the intradermal tuberculin test. The 3 animals negative to this test were not slaughtered, but were retained in the Williamson herd to serve as controls in future observations.

At the same time that the above 7 nonvaccinated heifers were tested with tuberculin by the intradermic method, 4 vaccinated heifers, of about the same age as the nonvaccinated controls, were selected at random from a group of 20, which, in 1926, had been vaccinated within 10 days of birth and which had been revaccinated 4 times since that year. The test on these 4 animals failed to show a degree of hypersensitiveness sufficient to cause a reaction definite enough to condemn any of the animals under the standards ordinarily used in routine testing. They were slaughtered for beef at the same time as the 4 controls.

None of the 5 vaccinated animals recorded in table 14A, showed any macroscopic evidence of tuberculosis. One, No. 236, did, however, carry live tubercle bacilli in one or more of its thoracic lymph nodes, since the guinea pigs injected with these apparently normal lymph nodes developed tuberculosis. The 4 heifers recorded in table 14A were selected at random from a vaccinated group in which all the animals were apparently in the same approximate state of health.

It will be noted that heifer 216 (table 14A) showed tuberculous lesions in the prescapular lymph node. The right prescapular node measured 80 x 40 x 30 mm and contained 6 caseous points, each 1 to

TABLE 14A

HEIFERS VACCINATED IN THE DEWLAP WITH 100 MG BCG AND MAINTAINED FROM BIRTH WITH A TUBERCULOUS HERD

No.	Born	Dates vaccinated	Remarks
264	Sept. 1, 1926	Sept. 4, 1926 Mar. 25, 1927 Oct. 18, 1927 July 18, 1928 July 15, 1929	Calved Feb., 1929. Drowned accidentally Aug. 14, 1929. No evidence of tuberculosis found, except three vaccination abscesses in the dewlap. Guinea pigs, inoculated from dewlap abscess and cervical, mesenteric, and thoracic lymph nodes, were negative for tuberculosis at 3 months.
268	Sept. 1, 1926	Sept. 1, 1926 Mar. 25, 1927 Oct. 18, 1927 July 18, 1928 July 15, 1929	Calved Feb., 1929. Negative to intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except eight 15-mm abscesses in the dewlap. Guinea pigs inoculated with cervical and thoracic lymph node tissue were negative for tuberculosis at 10 weeks.
236	June 29, 1926	July 3, 1926 Mar. 25, 1927 Oct. 18, 1927 July 18, 1928 July 15, 1929	Calved Mar., 1929. Negative to the intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except 3 abscesses in the dewlap, each 50 mm. Guinea pigs inoculated from the apparently normal thoracic lymph nodes were positive for tuberculosis at 10 weeks.
216	May 23, 1926	June 3, 1926 Mar. 26, 1927 Oct. 18, 1927 July 18, 1928 July 15, 1929	Calved March, 1929. Negative to the intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except in the dewlap and adjacent prescapular lymph nodes (see page 00). Guinea pigs inoculated from the cervical thoracic and prescapular lymph nodes, were negative for tuberculosis at 10 weeks.
212	May 11, 1926	May 21, 1926 Mar. 21, 1927 Oct. 18, 1927 July 18, 1928 July 15, 1929	Calved Mar. 19, 1929. Negative to the intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except 3 walnut-sized abscesses in the dewlap. Guinea pigs inoculated from the cervical thoracic and gastrohepatic lymph nodes, were negative for tuberculosis at 10 weeks.

TABLE 14B

NONVACCINATED HEIFERS SERVING AS CONTROLS FOR HEIFERS LISTED IN TABLE 14A

No.	Born	Remarks
205	April, 1926	Calved March 1, 1929, after which symptoms of generalized tuberculosis appeared. Killed April 21, 1929. Massive tuberculosis of organs and lymph nodes of the thoracic and abdominal cavities. Acid-fast bacilli were abundant in uterus and bronchial tubes.
203	April, 1926	Slaughtered for beef, September 4, 1929. A mesenteric lymph node contained a calcareous nodule, 20 x 15 x 8 mm, and in another node near by was a 7-mm caseous area. Acid-fast bacilli were demonstrated and inoculated guinea pigs developed tuberculosis.
225	Jan., 1926	Slaughtered for beef, September 4, 1929. The left retropharyngeal lymph node was enlarged 3 times normal size and about one-third of its area was thickly studded with caseocalcareous nodules, from 5 to 15 mm in size, typical of tuberculosis.
219	May, 1926	Slaughtered September 4, 1929. Condemned as unfit for food on account of emaciation. Extensive cervical and thoracic tuberculosis.
473	April, 1926	Slaughtered for beef, September 4, 1929. The left bronchial lymph node was enlarged 6 times normal size and contained a caseocalcareous lesion, 15 mm in size, in which acid-fast bacilli were demonstrated.

2 mm in size. These were grouped in a space 10 x 7 x 5 mm. The left prescapular node measured 70 x 40 x 20 mm and contained 8 caseous points, ranging from 2 to 3 mm in diameter. These were grouped together near the surface of the node. Near by was a yellow caseous mass, 3 x 4 x 3 mm in size. Acid-fast bacilli were demonstrated in smears from the above lesions as well as in the pus of several small abscesses at the points of vaccination in the dewlap. It appeared that the BCG organisms injected into the dewlap had penetrated the adjacent lymph nodes. The fact that guinea pigs inoculated from the prescapular lymph nodes failed to develop tuberculosis supports the idea that the prescapular lesions were caused by the BCG organisms and not by ingested acid-fast bacilli.

The fact that none of the vaccinated animals (table 14A) showed any macroscopic lesions attributable to virulent tubercle bacilli, while all of their controls (table 14B) had definite lesions, strongly supports the claims of Calmette.

It has been customary to slaughter all male calves for veal at the age of 2 or 3 months. To date, about 200 have been so slaughtered by the superintendent of the ranch, who reports that no lesions resembling tuberculosis have been observed except in the previously mentioned cases. In addition, tissues from several of these calves were sent to Berkeley and portions were injected into guinea pigs with negative results.

For economic reasons the owner has wished to sell some of the vaccinated heifers. They have, however, been found unmarketable except for beef, because it is supposed that they will probably react if subjected to the tuberculin test.

Until there is an opportunity to make post-mortem studies on more of the heifers which have been on the farm since 1926, the only conclusions that the writers are able to draw from the use of BCG in this herd are as follows:

1. No apparent injury to the general health or milking efficiency of the heifers has been produced by the annual vaccination.
2. A comparison in 1929 of the vaccinated and nonvaccinated heifers born in 1926 shows that the vaccinated heifers are all in good condition, while some of the nonvaccinated have died of tuberculosis. Five vaccinated heifers born in 1926 were found apparently sound on autopsy in 1929, while an equal number of nonvaccinated controls were found to be tuberculous.
3. Since the vaccine causes animals to become temporarily reactors to tuberculin, the vaccinated heifers were rendered for a time unmarketable, except for beef.

4. The results already obtained in the Williamson herd, when considered in connection with the results of the controlled experiments at Berkeley, are a distinct addition to knowledge concerning immunity and resistance to tuberculosis. A continuation of the cooperation with Mr. Williamson has therefore been arranged.

OBSERVATIONS OF THE EFFECT OF BCG ON CATTLE NOT EXPOSED TO TUBERCULOUS INFECTION

To determine if BCG cultures may cause any injury to cattle is of fundamental importance and a necessary prerequisite to extensive vaccination trials. Particularly, it is necessary to find out if extensive tuberculosis may result directly from the vaccination. It has been demonstrated by investigation in various countries, as well as by Calmette's group, that local tuberculous lesions are produced in the tissues of animals at the points where the living BCG bacilli lodge. The majority of reports concerning this indicate that these local lesions do not progress to an extent that is permanently detrimental to health and that if the animals are premitted to live, the lesions eventually heal. Consequently, the Commission of Bacteriologists of the Hygiene Section of the League of Nations⁽²⁸⁾ reported in 1928 that BCG constitutes a harmless vaccine for cattle. On the contrary, Watson⁽⁷⁴⁾ has reported that passages of BCG from calf to calf resulted, in one instance, in the development of virulence sufficient to cause generalized tuberculosis in guinea pigs inoculated from the calves.

To date, 27 head of cattle in the nontuberculous herd belonging to the University at Berkeley have been subjected to injections of BCG in various ways. Following is a list of these trials:

1. Eight aged cows were injected in the dewlap with BCG, dose 100 mg. On slaughter after 72 days, no tuberculous lesions were found except the usual cold abscesses at the point of vaccination.

2. Fifteen calves born in 1926 and 1927, were vaccinated in the dewlap from 1 to 10 days after birth, and have been revaccinated annually since that time. To date (November, 1929), no injurious effect has been noted from the vaccine other than the local abscess produced in the dewlap. In one of these animals (No. 1111) after the second vaccination on February 12, 1927, the dewlap lesion gradually developed to such a size that it became an objectionable blemish. In September, 1928, it consisted of multiple intercommunicating abscesses, forming 3 tumors having respective diameters of 150, 80 and 70 mm. The contents consisted of semi-solid caseocalcareous, necrotic tissue.

On microscopic examination, large numbers of acid-fast bacilli were found mixed with leucocytes and amorphous, necrotic debris. Cultures on serum egg and other media remained sterile. Four guinea pigs inoculated with the pus were killed after 6 months and found to be in excellent condition. No lesions of tuberculosis were found in them.

An attempt was made to reduce the size of the abscesses in calf No. 1111 by free incisions, drainage, and the liberal use of tincture of iodine, without much effect; but, after several injections of hexyl-resorcinol, the discharges ceased and healing resulted.

On December 19, 1928, the animal was revaccinated in the dewlap with 100 mg of BCG. In August, 1929, the 3 swellings in the dewlap measured respectively 110, 110, and 80 mm.

3. Three calves, aged 2 months each, received the relatively massive doses, 1 to 4 grams, distributed in smaller amounts at various points

TABLE 15
THE RESULTS OF INTRAVENOUS INJECTIONS OF BCG IN CATTLE AND SWINE

No.	Sex and species	Age when injected	Dose of BCG, mg	Remarks
82	Steer.....	5 months.....	100	Temperature remained normal. Butchered 30 days after injection of BCG. Tissues were apparently normal throughout on macroscopic examination except that on close scrutiny the lungs were seen to be studded throughout with glass-like nodules, pin point to 0.5 mm in size and occasionally a larger nodule (1 mm) was observed. A histological study showed the lungs to be thickly studded with foci containing acid-fast bacilli (figs. 15 and 16). Guinea pigs injected with lung and lymphatic tissue remained normal.
94	Steer.....	2 months.....	.50	Temperature irregular for 4 weeks; otherwise the animal appeared normal. Slaughtered 4 months after injection. Tissues normal throughout. Lungs normal histologically. Guinea pigs injected with lung and lymphatic tissue remained normal.
1130	Heifer.....	2 weeks.....	500	Temperature irregular for 4 weeks. The animal grew slowly and was unthrifty for 6 months; otherwise she has remained normal. Still living; age 2 years.
1110	Heifer.....	21 months	500	Temperature high on 2nd and 3rd days and on 16th to 24th days after injection. Otherwise she has remained normal. Died from dystocia, aged 2½ years. No indications of tuberculosis found.
A	Gilt.....	3 months.....	500	Irregular temperature between 2nd and 28th day after injection. The day after injection partial paralysis of the rear limbs developed, from which recovery was slow, and the animal was still slightly lame on slaughter 13 weeks after the BCG was injected. Apparently normal throughout on macroscopic inspection except that on close examination in a strong light the lungs were seen to be studded with white nodules up to 0.5 mm in size. A histological study of the lungs showed that they were studded with necrotic foci containing acid-fast bacilli (figs. 17, 18). Guinea pigs inoculated with lung and lymphatic tissue remained normal.

under the skin. Cold abscesses formed at the points of injection, but the animals have remained in good general condition and are being held under observation.

4. A study has been made of the effects of intravenous injections of BCG into 4 calves in a tuberculosis-free environment. These results are listed in table 15 and serve as a control on the infection experiments described in table 3, as well as showing the reaction to such injections in animals protected from virulent tuberculous infection.

Discussion of Table 15.—No permanently injurious effects resulted from the intravenous injection of massive doses of BCG. However, in some animals a disturbance occurred which was evidenced by irregularities in the temperature for a few weeks.

The lung nodules in steer No. 82 and swine *A* consisted of a central mass of acid-fast bacilli, surrounded by a wall of connective tissue. See figures 15-18.

Apparently the lung lesions are resorbed in a few weeks because no trace of lung abnormality was found other than a slight increase in the interlobular connective tissue in animals killed after an interval of more than 4 months.

HYPERSENSITIVENESS IN VACCINATED CALVES

During the experiments at Berkeley, it has been observed that a rise of temperature may occur in calves soon after revaccination subcutaneously with BCG. The fever begins in from 2 to 6 hours after the injection and ranges between 104° and 106° F for 8 to 14 hours. The temperature curve is similar to the thermal reaction caused by tuberculin and seems to have no relation to the fever which occurs in calves 2 or 3 weeks after the intravenous injection of BCG.

No temperature reactions immediately after the subcutaneous injection of the vaccine have been observed at this Station in tuberculosis-free calves vaccinated for the first time, and we believe that when such reactions do occur, they indicate that the animal has previously been infected by tubercle bacilli or organisms of similar antigenic effect.

Figure 3 shows the temperatures recorded after the vaccination of 3 calves. The records of these animals have been selected for graphic presentation in chart form because they represent three types as explained in the legend under figure 3.

During 1926 and 1927 in accordance with the recommendations of Calmette, the use of tuberculin was avoided when the calves were to

be exposed to virulent infection, although, according to the experiments of Goldenberg of the Ukrainian Commission as reported by Tzeknovitzer,⁽⁷²⁾ such a precaution is probably unnecessary.

Studies of the effect of tuberculin on the vaccinated calves which were protected as much as possible from exposure to virulent bacilli have been made at the California station. In the 20 cases studied, the subcutaneous injection of 100 mg of BCG was always followed by the development of a sensitiveness to tuberculin as evidenced by intradermic and ophthalmic reactions. In the majority of the vaccinated calves, a thermal reaction followed the subcutaneous injection of 500 mg of tuberculin O. T.

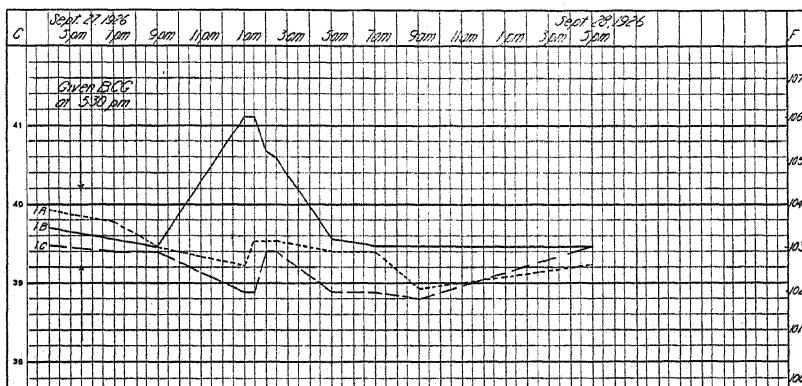


Fig. 3. Temperature chart showing fluctuations in temperature after the injection of BCG into the dewlap.

Calf 1A received 40 mg. It had previously received 50 mg on June 6, 1926, and a vaccine abscess had formed, discharged, and healed before September 27, 1926.

Calf 1B received 40 mg. It had previously received 50 mg on June 6, 1926. An abscess had formed but did not open. On September 27 it measured 27 x 27 mm.

Calf 1C received 50 mg. It had not been vaccinated previously.

The surgical removal of the cold abscess in the dewlap of calf No. 1114 (fig. 8, p. 388) was followed by a gradual reduction in local sensitiveness to tuberculin, although slight traces of sensitiveness to ophthalmic, intradermic, and subcutaneous tuberculin tests persisted for 18 months.

King and Park⁽⁴⁰⁾ failed to obtain any indication of increased sensitiveness to tuberculin in guinea pigs and calves that had been fed small doses of BCG.

In order to determine if the feeding of a massive dose of BCG does cause hypersensitiveness, 2 calves at the California station at Berkeley were each given by mouth 1 gram of BCG suspended in physiological

sodium chloride solution. Calf No. 472 was 72 days old at the time the BCG was fed and calf No. 1022 was just born and had never nursed. Sixty days later the calves were each given intradermally 0.2 cc of 100 per cent tuberculin O. T. This provided as strong a dose as is ordinarily used to elicit thermal reaction and at the same time subjected the calves to intradermic test with several times the ordinarily used dose of tuberculin. No thermal reactions were observed. Observations at 60, 84, and 132 hours showed no positive intradermic reactions, although both calves developed slight subcaudal thickenings, the thickenings in calf No. 1022 being slightly the larger ("P" according to the U. S. Bureau of Animal Industry Standard). At the 84th hour observation a sensitizing ophthalmic disc was placed in one eye of each animal and 72 hours later a test dose disk was given each calf. No reactions developed during a 12-hour observation. There was no thermic reaction. Three pre-injection and 10 post-injection temperatures were taken. The latter were started $6\frac{1}{2}$ hours after injection and were taken at 2-hour intervals. Calf No. 472 was butchered at 140 days of age. No indications of tuberculosis were found. Calf No. 1022 is still living and in good condition.

CONTROL TESTS OF BCG CULTURES ON SMALL ANIMALS

Guinea Pigs.—In all, 106 guinea pigs have been used to test the purity and harmlessness of the cultures as required by Calmette. The dosage has ranged from 2 to 100 mg given in various ways. Ninety of these animals have been killed from 2 to 13 months after vaccination. The remainder are being held for observation.

Intramuscular and subcutaneous injections into guinea pigs have usually resulted in the formation of local abscesses which, as a rule, rupture and heal after a few weeks without affecting the general health of the animal. Thirteen months after injection one of the guinea pigs still had about a minim of pus at the point in the thigh where 25 mg had been injected. The pus from these discharging abscesses contained acid-fast bacilli, but at this station subinoculations of such pus into other guinea pigs have thus far always given negative results.

Intraperitoneal injections of 2 to 50 mg into guinea pigs were followed by well-marked adhesions or purulent lesions or both in the abdominal cavity. Such lesions have been demonstrated in all the animals killed 2 to 13 months after inoculation, but the subinoculations thus far have been negative.

Rabbits.—Thirteen rabbits have been injected intramuscularly or subcutaneously with doses of BCG ranging from 20 to 100 mg. Local abscesses formed which were usually larger and more persistent than in the guinea pigs. This does not necessarily indicate that rabbits are more susceptible than guinea pigs to specific pathogenicity of BCG. It may be accounted for by the fact that they are apt to develop relatively larger local lesions than guinea pigs when injected intramuscularly or subcutaneously. Besides, the work of Coulaud⁽²⁹⁾ and others who have injected rather large doses of BCG intravenously into rabbits does not suggest that rabbits are particularly susceptible to BCG, since no generalization of disease is produced; in fact, complete healing has taken place in their cases after several months. One rabbit at the California station which had been injected subcutaneously inside the right thigh with 20 mg was killed after an interval of 105 days. A 30-mm abscess was found between the muscles of the thigh. The liver also contained a white nodule the size of a pea. Acid-fast bacilli were found in smears from both these lesions.

Swine.—In an attempt to determine if vaccination has any immunizing effect on swine, injections of BCG have been made into pigs at various ages ranging from 1 day to 6 months old. To date, 45 swine have been vaccinated in various ways, intravenously, subcutaneously, intramuscularly, intradermally, and by mouth. The results are not ready for publication, but as yet no indication has been found that the BCG cultures at this Station are capable of producing generalized or fatal tuberculosis in swine. The lesions are local in character and resemble those produced in calves.

Comment.—The fact that the intraperitoneal injection of BCG into guinea pigs and rabbits often causes rather extensive caseous lesions has been the basis of some criticism of the early statements of Calmette as to the harmlessness of BCG. However, it would seem that abdominal lesions resulting from such intraperitoneal injections are of little significance in solving the problem of the possible harmfulness of the subcutaneous and oral vaccination procedures recommended by Calmette.

On the other hand, the cases of generalized tuberculosis in guinea pigs after injection with dissociated BCG cultures reported by Petroff, Branch, and Steenken⁽⁶¹⁾ are of great interest. In their work, however, the technique recommended by Calmette was not followed; instead a deliberate attempt was made to develop virulent strains by dissociating the BCG cultures into R and S types of colonies by growing the bacilli on gentian violet egg media. Chiari, Nobel, and Sole⁽²⁵⁾ also have reported the cultivation from BCG of a strain virulent for guinea pigs.

Watson^(74, 75, 76) was one of the first to question the inoffensiveness of BCG. He reports results from the injection of 134 guinea pigs with BCG as follows:

In 92, or 68.7 per cent, no lesions of tuberculosis were discernible after a duration period averaging 295 days; in 30, or 22.3 per cent, early, slight or localized lesions were found after an average duration of 290 days, and in 12, or 9 per cent, typical generalized tuberculosis developed after an average duration of 309 days.

Watson also questioned the alleged benign character of tuberculous lesions as found in guinea pigs following BCG inoculation and developing in the course of many months. In some cases he reported inoculations made from such so-called benign lesions produced tuberculosis, and, furthermore, serial passage from guinea pig to guinea pig was reported to be productive of typical tuberculosis.

Doyle⁽³¹⁾ has claimed that certain parts of Watson's work are open to criticism, but nevertheless, merit immediate repetition in other laboratories.

In experiments at the United States Experiment Station, Bethesda, Maryland, Schroeder and Crawford⁽⁶⁵⁾ have reported that in one lot of 36 guinea pigs injected with BCG, 1 died showing lesions of generalized tuberculosis. None of the other 35 were affected. It was not decided whether the 1 guinea pig of this series that died of tuberculosis contracted the disease as a result of the inoculation with BCG or whether this was a case of 'spontaneous' tuberculosis. They stated that when guinea pigs are kept in the same house with tuberculous guinea pigs, there is always a possibility of 'spontaneous' tuberculosis. Sewall⁽⁶⁷⁾ has called attention to the occurrence of so-called 'spontaneous' tuberculosis in guinea pigs and the possibility of the introduction of infection to them on contaminated feed.

The observations of Gerlach,⁽³⁵⁾ King and Park,⁽⁴⁰⁾ Woodruff and Gregory,⁽⁷⁸⁾ and many others, as well as the results at the California station, support the contention of Calmette that no increase to high virulence has yet been proved to occur in BCG cultures maintained on ox-bile potato.

At the California Agricultural Experiment Station cultures of BCG have been transplanted on potato in 5 per cent glycerine broth since 1926 and a parallel series has been carried on the same medium with each tenth and eleventh transplant on ox-bile potato containing 5 per cent glycerine. In attempts to dissociate the cultures carried in this way, it was possible at the California station to obtain from the former series variants simulating both R and S types of colonies as described by Petroff, while in the latter series carried according to

Calmette's recommendations, these have not been observed. Guinea pigs inoculated with the S type of colony showed no significant variations from those inoculated with the R type. A slightly enlarged spleen, found in the guinea pig inoculated with the S type, was reinoculated into 2 guinea pigs. These animals showed no evidence of disease when slaughtered at the end of 75 days. One rabbit, inoculated intravenously with about 50 mg of the S type growth, when killed after an interval of 3 months, showed the lungs to be studded with 1 to 2-mm nodules, containing acid-fast bacilli. This lung tissue was reinoculated intramuscularly into 2 guinea pigs. These animals were slaughtered at the end of 75 days and showed no evidence of disease. A rabbit inoculated in a similar way with the R type of growth was in poor condition on autopsy after an interval of 3 months. The lung contained 7 necrotic spots, consisting of creamy pus, 1 to 2 mm in size. The spleen was also slightly enlarged. No acid-fast bacilli or other indications of tuberculosis were demonstrated.

DISCUSSION AND SUMMARY

Two hundred and eighty-two calves less than 10 days of age, and 15 older cattle were treated with cultures or transplants of cultures received from Calmette. One hundred and ninety-two of the calves were in an extensively tuberculous dairy herd, where vaccinations have been made for 3 years under partially controlled conditions. The remaining 90 calves were from herds free from reactors to the tuberculin test. These have been maintained with an equal number of nonvaccinated controls at the California Agricultural Experiment Station at Berkeley and Davis.

Thirty of the 90 head were used to test the possibility of any injurious effects of BCG on cattle in a presumably tuberculosis-free environment, and the remaining 60 were used to test the immunizing or resistance-producing power of the vaccine by exposing them to tuberculous infection under experimental conditions.

A summary of the results of experimental infection of the 60 head of vaccinated cattle and of 62 of the nonvaccinated controls is given in table 16. This table also contains the results from the infection of 2 calves treated with heat-killed tubercle bacilli and 2 treated with nonvirulent acid-fast bacilli from a culture isolated from bovine lymphangitis.

The results under controlled conditions listed in table 16 indicate that the use of BCG confers upon cattle a definite resistance to tubercle

TABLE 16
SUMMARY FROM PRECEDING TABLES OF RESULTS IN VACCINATED AND CONTROL CALVES

Number of table in preceding text summarizing	Number and sex	Method of vaccination	Form and frequency of infection	Autopsy findings	Conclusions
3	2 steers	100 mg BCG in dew-lap.	2 mg bovine tubercle bacilli intravenously.	1, slaughtered 4½ months after infection, had no lesions, but guinea pigs became infected from the apparently normal lymph-node tissue. The other calf, slaughtered 10 months after infection, had slight but widely distributed lesions.	The BCG subcutaneously gave sufficient resistance to prevent the fatal effects of intravenous infection.
3	2 steers	50 mg BCG intravenously.	2mg bovine tubercle bacilli intravenously.	1 died of tuberculous pneumonia 51 days after infection. The other remained apparently normal, but, on slaughter 110 days after infection, was found to have extensive thoracic lesions.	The BCG intravenously apparently retarded fatal results in 1 and prevented fatal effects in the other calf.
4	2 steers	1 gram heat-killed tubercle bacilli intravenously.	2mg bovine tubercle bacilli intravenously.	Died of miliary tuberculosis in 29 and 31 days, respectively, after infection.	Vaccination with killed tubercle bacilli had no apparent effect.
3	4 steers	Controls.	2 mg bovine tubercle bacilli intravenously.	Controls died of miliary tuberculosis in from 20 to 34 days after infection.	
3	2 steers	100 mg BCG in dew-lap.	50 mg bovine tubercle bacilli injected subcutaneously.	On slaughter after 2½ and 4 months, respectively, both were found to have well-marked lesions at point of infecting injection and slight lesions in certain lymphatic tissues.	An evident resistance was conferred by the vaccine.
3	2 steers	Controls.	50 mg bovine tubercle bacilli injected subcutaneously.	Developed advanced clinical tuberculosis. On slaughter after 2 months, both had generalized tuberculosis.	

6	4 steers	100 mg BCG in dew-lap.	6 feedings.	1 calf had extensive mesenteric and slight retropharyngeal lesions; 2 had slight mesenteric lesions and in 1, no lesions were found.	The lesions in the calves vaccinated with BCG were less pronounced than in the respective controls, and the lesions in the calves treated with acid-fast bacilli from a bovine skin lesion culture were more pronounced than in the controls.
6	4 steers	Controls.	6 feedings.	All 4 had extensive caseocalcification of 1 or more groups of lymph nodes.	
6	2 steers	100 mg acid-fast bacilli from a bovine skin lesion.	2 feedings.	Tuberculous pneumonia from aspiration in 1 and extensive lymphatic caseocalcification in the other.	
6	2 steers	Controls.	2 feedings.	Extensive lymphatic caseocalcification in 1 calf, and retropharyngeal caseation in the other.	
7A	19 steers	100 mg BCG in dew-lap.	10 feedings.	Of 14 calves infected, 35 or more days after vaccination, 3 had well-marked lesions, 6 had slight lesions, and in 5 no lesions were found. One calf died of gastritis too soon to justify consideration.	In general the lesions in the calves infected 35 days or more after vaccination were less extensive than in the controls. The lesions in 4 calves infected within 28 days after vaccination were widely distributed but not large in size.
7B	19 steers	Controls.	10 feedings.	10 had extensive, 4 had well-marked, and 3 had slight lesions. In 1, no lesions were found, but the guinea-pig test was positive.	
8A	10 steers	100 mg BCG in dew-lap.	30 feedings.	1 calf had extensive caseation of mesenteric nodes; 1 had well-marked lesions in the spleen and slight lesions in the mesentery; 1 had well-marked mesenteric lesions only; 1 had well-marked mesenteric, cecal, and cervical lesions; and 5 had slight caseous lesions. In 1, no tuberculous lesions were found.	The lesions in the vaccinated calves were less extensive than in their respective controls in 8 animals; in 2, the lesions were about equal to the controls.
8B	10 steers	Controls.	30 feedings.	5 had well-marked and the other 5 extensive lesions.	
9A	5 steers	100 mg BCG in dew-lap.	36 feedings. The last 6 were massive.	4 had slight, but widely distributed, lesions. The other had well-marked cervical and slight mesenteric and ileocecal lymph-node lesions.	The lesions in 4 of the vaccinated calves were less extensive than in their respective controls. In the other vaccinated calf, the lesions were more numerous and widely distributed but smaller in size than in its control.
9B	5 steers	Controls.	36 feedings. The last 6 were massive.	3 had extensive and 2 had well-marked lesions.	

TABLE 16—(Concluded)

Number of table in pre- ceding text summarized	Number and sex	Method of vaccination	Form and frequency of infection	Autopsy findings	Conclusions
10A 2 steers	100 mg BCG in dew- lap.	Fed on milk of tu- berculous udder, cow No. 600.	I had slight mesenteric and well-marked iliocecal lymph- node cæsation. The other calf had no demonstrable tuberculous lesions.	The controls were more tuberculous than the vaccinated.	
10B 2 steers ^s	Controls.	Fed on milk of tu- berculous udder, cow No. 600.	Both had well-marked tuberculous lesions.		
10A 2 steers	100 gm BCG in dew- lap.	Fed on milk of tu- berculous udder, cow No. 2171.	I had extensive cæsation of iliocecal nodes and well-marked lesions elsewhere; I died of intercurrent causes 8 months after infection and the only tuberculous lesion found was a slight retropharyngeal involvement.		
10B 2 steers	Controls.	Fed on milk of tu- berculous udder, cow No. 2171.	Both died of generalized tuberculosis.		
11A 3 steers	100 mg BCG intra- venously.	Feeding.	2 had slight abdominal lesions; in the other no lesions were found, but guinea pigs injected with its cervical and mes- enteric lymph nodes became tuberculous.		
11A 6 steers	Massive doses of BCG by mouth.	Feeding.	I had well-marked tuberculous lesions; 3 slight lesions local- ized in the retropharyngeal lymph nodes; I had widely dis- tributed lesions; and in 1, no lesions were found but guinea pigs injected with its cervical nodes became tuberculous.		
11A 3 steers	100 mg BCG intra- eutaneously.	Feeding.	I had well-marked and 1 had slight tuberculous lesions. The 3rd died of intercurrent gastritis too soon to be of use.		
11B 12 steers	Controls on above 12 calves.	Feeding.	10 had extensive tuberculosis and in the other 2 the lesions were well-marked. In all 12 controls the lesions were more pronounced than in any of the corresponding vaccinated calves.		

bacilli. As already pointed out by Zwick and Witte⁽⁸⁰⁾ in a summary of the literature, resistance against artificial infection in this way is of little significance in answering the question of the practical value of the vaccine. This may be true, but natural infection is so beset with variables that definite conclusions from experiments under farm conditions seem unreliable to the writers unless confirmed by controlled experiments under field laboratory conditions. It is hoped that a continuation of the investigations on the cattle in the dairy herd belonging to Mr. H. D. Williamson will add materially to the data necessary for a definite answer.

For purposes of a comparative grouping of the autopsy findings in the 126 animals listed in table 17, a lesion was arbitrarily classed as slight, well-marked, or extensive in accordance with the standard listed in the footnote of table 6. When tabulated under such groupings, the results expressed in table 17 were obtained.

TABLE 17
RELATIVE EXTENT OF THE TUBERCULOUS LESIONS IN CALVES

Type of lesions	Vaccinated*	Controls	Supplementary controls†
Extensive.....	7‡	40‡	5†
Well-marked.....	13	16	1
Slight.....	27	3
None visible.....	13	1
Total.....	60	60	6

* Nine of the calves were subjected to infection within 30 days of vaccination (see tables 7A, 9A, and 10A). If these 9 are eliminated, the tabulated results for vaccinated calves will be changed as follows:

Extensive..... 6 Well-marked..... 10 Slight..... 23 None visible..... 12

† In addition to the 60 controls, use was made of 6 calves as supplementary controls; 2 of these had each been previously treated with an intravenous injection of 1 gram of heat-killed tubercle bacilli; each of 2 others had received a subcutaneous injection of 100 mg of nonvirulent acid-fast bacilli in culture from bovine lymphangitis; and 2 were unvaccinated.

‡ Includes 9 cases of fatal tuberculosis in controls and 2 in vaccinated calves. These 2 animals had been vaccinated in the jugular vein and 4 months later infected through the same channel.

It is evident that the subcutaneous vaccination afforded protection against the fatal effects of intravenous and subcutaneous infection with cultures of virulent bovine tubercle bacilli. On the other hand, the relatively slight differences between the number of lesions in the vaccinated and the control calves which had been subjected to repeated infections through the mouth indicated that no measurable resistance was afforded by the vaccination to the entrance into the tissues of virulent tubercle bacilli, but the tissue damage resulting from such invasion was in most cases less in the vaccinated than in the control calves.

The most marked contrasts between the vaccinated and control calves infected by mouth occurred in the groups described in tables 10A and 10B, in which the tuberculous infection received by the 4 calves 47, 48, 147, and 148, from the milk of the tuberculous udder of cow No. 2171, was either very virulent or very massive, inasmuch as both controls died of tuberculosis at 62 and 162 days respectively. Vaccinated calf No. 47 remained in good general condition and was butchered 94 days after the first infecting feeding. Widely distributed tuberculous lesions were found, but they were not extensive enough to affect the general condition of the animal. Vaccinated calf No. 48 remained in good general condition until death by accident 246 days after infection. Only slight lesions of tuberculosis were found.

The failure of the subcutaneous vaccination to produce complete immunity to tuberculous feeding infection at the California station led to some preliminary trials on calves at Berkeley in which BCG was introduced into the body through intravenous, intradermic, and oral channels. Tables 11A and 11B show that these methods also apparently conferred some resistance to feeding infection, but not appreciably greater than that conferred by subcutaneous vaccination. Against the fatal effects of intravenous infection, the subcutaneous method of vaccination was more effective than the intravenous, as judged by the results tabulated in table 3.

A comparison of the autopsy results on the various groups of control calves indicates that the prolonged feeding of massive doses of tubercle bacilli does not usually result in the production of more extensive lesions than when the feeding is stopped at the end of 10 feedings. It may be seen from tables 7B, 8B, and 9B that 10 of the 19 control calves which received 10 feedings had extensive lesions, while only 4 cases of extensive tuberculosis occurred in the 15 control calves which received 30 to 36 feedings. It should be noted that the latter 15 calves had received their first 10 feedings from the same mixtures that were given the first mentioned group of 10 animals.

These results support the generally accepted theory that the lesions resulting from the first tubercle bacilli to invade the body tend to create a resistant power in the tissues against future tuberculous infection. The results, however, are not entirely in accord with the assertions of Calmette⁽⁷⁾ that only fresh tuberculous infection, occurring again and again at short intervals, is able to overcome the natural barrier of the mesenteric lymph nodes.

The fact that no macroscopic lesions of tuberculosis could be found in 5 vaccinated heifers (table 14A) which were kept exposed to infection for 3 years at the Williamson Ranch, while tuberculous lesions

were found in 5 nonvaccinated heifers of approximately the same age on that ranch tends to support Calmette's claims.

The writers⁽³⁸⁾ have observed that, under conditions prevailing in certain parts of California, it is not difficult in tuberculous herds to rear heifers free from tuberculosis until they calve for the first time. Owners are then often under the necessity of introducing the disease-free heifers into the infected milking herds. A possible use for BCG may be found in the vaccination of such heifers. In the writers' opinion, if the resistance conferred is sufficient to prevent the development of open lesions, the problem of reducing the incidence of tuberculosis to a point where eradication measures may be economical in such herds will have been solved. In view of this, experiments are being planned at the California station to further test out the safety and practicability of vaccinating tuberculosis-free heifers during their first pregnancy and continuing their revaccination annually after they have been introduced into herds of tuberculous cattle.

CONCLUSIONS

The subcutaneous vaccination of cattle with 100-mg doses of BCG conferred sufficient resistance to protect against the fatal effects of intravenous or subcutaneous injections of virulent tubercle bacilli.

In feeding trials with virulent tubercle bacilli, the vaccinated cattle showed less extensive lesions, as a rule, than the unvaccinated. The prolongation of the feeding of calves with massive doses of virulent tubercle bacilli apparently had no effect in increasing the number or size of the tuberculous lesions found on autopsy 4 to 12 months later. The calves which received from 2 to 10 infecting feedings had just as extensive lesions, on the whole, as those animals which were fed from 20 to 26 additional doses of much larger numbers of virulent tubercle bacilli. This was observed in both the vaccinated and nonvaccinated groups.

Feeding infection experiments with calves following the intradermic, intravenous, or oral administration of BCG indicated that these methods of vaccination are not superior to the subcutaneous.

The resistance afforded by the vaccine was not sufficient to always prevent the penetration of the walls of the alimentary tract by virulent tubercle bacilli. In most cases this induced a caseation of the cervical and mesenteric lymph nodes. The chief protective effect of BCG seems to be in retarding the extension of tuberculous processes occurring from infection received subsequent to vaccination.

Apparently the subcutaneous method of vaccination has furnished protection against the development of clinical cases of tuberculosis in heifers in a tuberculous dairy herd.

The nonprogressive tuberculous changes or the local vaccination lesions, or both, will render the majority of vaccinated cattle hypersensitive for a time to the intradermal injection of tuberculin, making such animals temporarily unmarketable in California except for beef.

BCG appears to be somewhat effective in protecting against a fatal¹⁰ termination of massive infection.

The resistance to tuberculosis conferred by subcutaneous, intravenous, intradermic, or oral methods of administration of BCG, as used at the California station, is not sufficient to justify the use of the vaccine on cattle where measures designed to eradicate tuberculosis in cattle are being successfully carried out. On the other hand, in countries or localities where control measures are proving ineffective or where eradication seems to be hopeless for many years in the future, the vaccine may eventually be found of economic value to cattle owners by preventing the occurrence of extensive or fatal lesions and by limiting the spread of the disease.

Observations of the effect of BCG in cattle, swine, rabbits, and guinea pigs at the California Agricultural Experiment Station have thus far failed to detect the production of any lesions which could be proved to be virulent by reinoculation.

¹⁰ The observation that subcutaneous vaccination with BCG protects against the fatal effects of tuberculosis is supported by unpublished results obtained in the routine vaccination of monkeys kept at the George Williams Hooper Foundation for Medical Research, University of California, under the direction of K. F. Meyer. The vaccine used on the monkeys was made at the California Agricultural Experiment Station and was similar to that employed in the cattle experiments.

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FIGURES 4-18



Fig. 4

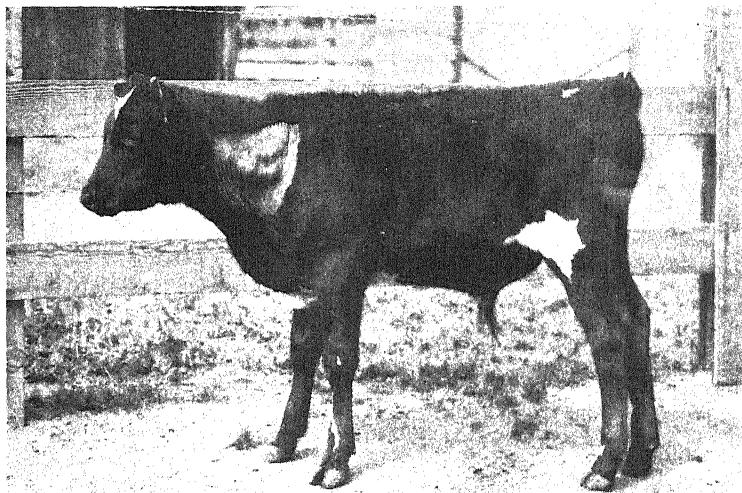


Fig. 5

Figs. 4 and 5. Appearance of the tuberculous lesions in the necks of vaccinated calves 5 and 6, 60 days after the subcutaneous injection of 50 mg of virulent tubercle bacilli into the left side of the neck. Compare with figures 6 and 7.

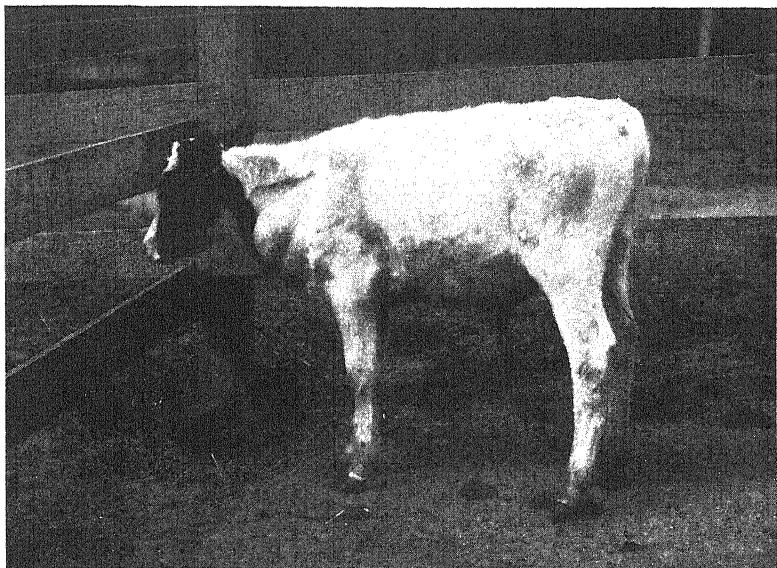


Fig. 6

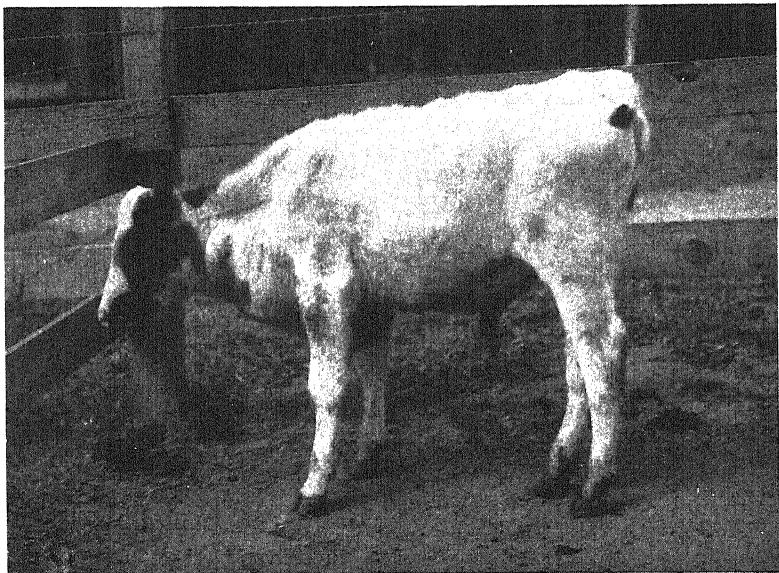


Fig. 7

Figs. 6 and 7. Nonvaccinated control calves 105 and 106, showing appearance 60 days after the injection of 50 mg of tubercle bacilli.



Fig. 8. Abscess in the dewlap (calf No. 1114) typical of the usual effect from the injection of 100 mg of BCG. Eight months after injection, on the day the above photograph was taken, the abscess and its surrounding tissues were removed surgically. Intradermic hypersensitivity to tuberculin gradually decreased in this calf, although after 18 months the animal was found to be still slightly hypersensitive to ophthalmic and intradermic applications of tuberculin.



Fig. 9. Section of the left fore quarter of the udder of cow No. 600, showing extensive tuberculous lesions. The right rear quarter of this cow was similarly affected. The milk from this cow was used for infection feedings on vaccinated calves 45 and 46 and nonvaccinated control calves 145 and 146, and resulted in moderate tuberculous lesions in all four. See tables 10A and 10B.



Fig. 10. Appearance of the udder of cow No. 2171 on November 23, 1927. Tuberculous induration had distended the right rear quarter so that the teat of that quarter was shortened and displaced toward the left and to the front. The milk from this cow was used for infection feedings on vaccinated calves 47 and 48 and nonvaccinated control calves 147 and 148, and resulted in well-marked or extensive infection in all four. The vaccinated calves remained in good general condition. The nonvaccinated controls died of tuberculosis. See tables 10A and 10B.

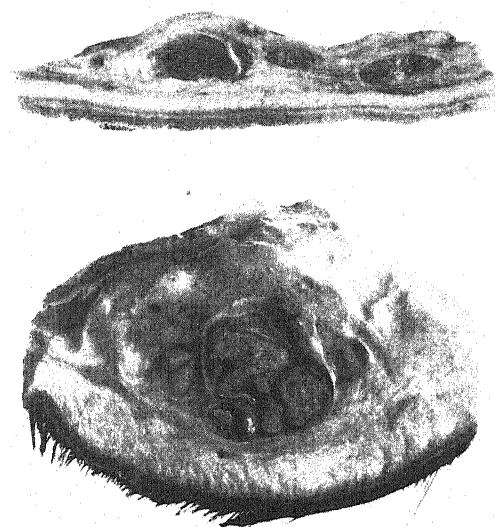


Fig. 11. Typical lesions caused by the injection of 100 mg BCG into the dewlaps of calves. Cross sections actual size. Upper from calf No. 38, removed at autopsy 246 days after vaccination (see table 8A). Lower from calf No. 31, removed 447 days after vaccination (see table 8A). Acid-fast bacilli were abundant in smears from these lesions.

See page 393 for illustrations.

Fig. 12. Transections of mesenteric and ileocecal lymph-nodes, showing caseous and caseocalcareous areas. The normal tissues have been partially trimmed away. This illustration shows in natural size all of the tuberculous lesions found in vaccinated calf No. 38. Compare with lesion symbols for this calf in table 8A.

Fig. 13. Transections of caseous and caseocalcareous lymph nodes from control calf No. 138. Left to right, top row, left retropharyngeal, right prescapular, right retropharyngeal; second row, anterior and median mediastinal and three mesenteric; third row, five mesenteric; lowest row, two mesenteric, one colic, one gastrohepatic. The normal tissue has been partially trimmed away from each node shown. No lesions were found in calf No. 138 other than those shown in figures 13 and 14, except in the retropharyngeals which were studded throughout as shown on the cut surfaces in figure 13. (Natural size.)

Fig. 14. Appearance, natural size, of a caseous nodule in a Peyer's patch, ileum of calf No. 138. The mucous membrane has been removed to show the caseous material beneath. Acid-fast bacilli were demonstrated to be present in large numbers and inoculated guinea pigs developed tuberculosis.

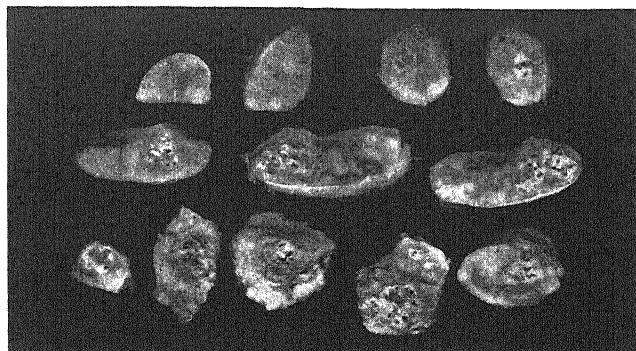


Fig. 12

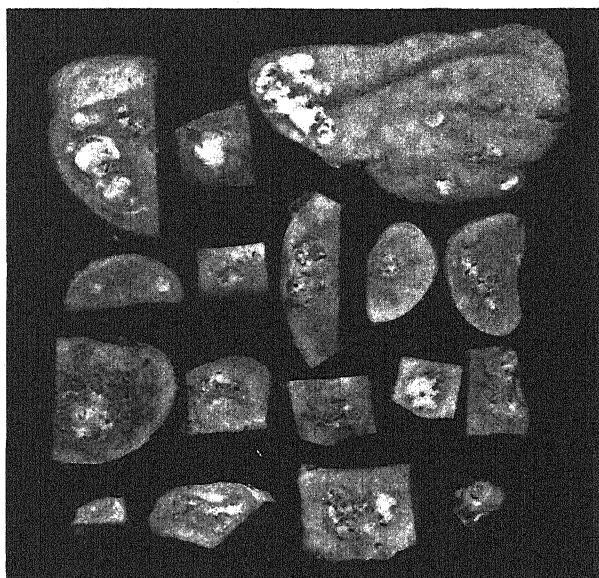


Fig. 13

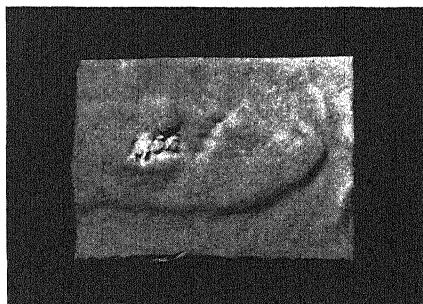


Fig. 14

See page 392 for explanation of above.

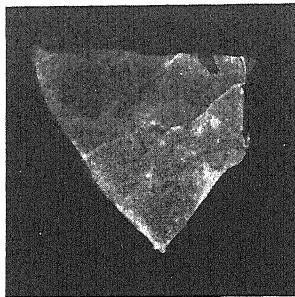


Fig. 15

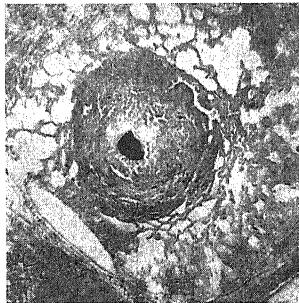


Fig. 16



Fig. 17

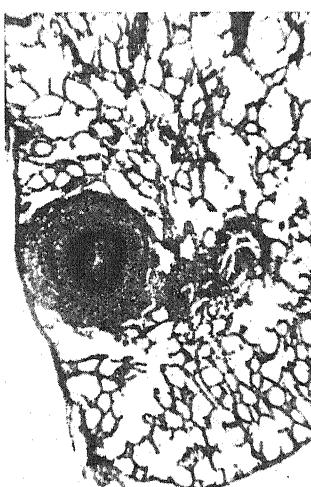


Fig. 18

Fig. 15. Appearance of the surface of a portion of the lung, natural size, pleura intact, of calf No. 82 (see table 15). The white specks are lesions resulting from the intravenous injection of BCG. Calf No. 82 was butchered while in apparently good health 30 days after the injection of 100 mg of BCG into a jugular vein.

Fig. 16. Appearance of a section through one of the nodules in the lung of calf No. 82. The black mass in the center consisted of clumped acid-fast bacilli. Staining: Ziehl-Neelsen and methylene blue. (Enlarged 20 x.)

Fig. 17. Appearance of the surface of the lung, pleura intact, of swine A, showing a typical nodule caused by BCG (see table 15). (Enlarged 5 x.)

Fig. 18. Appearance of a section through one of the nodules in the lung of swine A. The dark mass in the center consisted chiefly of acid-fast bacilli. Staining: Ziehl-Neelsen and methylene blue. (Enlarged 40 x.)

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SPIROCHETES AS THE ETIOLOGICAL FACTOR IN CERTAIN SPECIFIC NECROSES AND HYPERPLASTIC FORMATIONS IN SWINE

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The high percentage of young swine on the Pacific Coast of the United States affected with rhinohyperplasia² and scirrhous cord (neoplastic formations in the scrotum following castration) has been the incentive for some investigations, which are herewith reported.

Published observations concerning a form of rhinitis in swine called 'snuffling sickness' appeared in the German veterinary literature as early as 1860. Haubold,⁽¹⁾ Harms,⁽²⁾ Wulff,⁽³⁾ and Ostertag,⁽⁴⁾ considered the cause to be a rachitic swelling of the ethmoid and sphenoid bones. According to Schell,⁽⁵⁾ sarcomatous growths produced the symptoms, while Haubner⁽⁶⁾ considered it a form of tuberculosis, and Damman⁽⁷⁾ believed some cases to be caused by actinomyces, and others by abnormal development of the nasal bones. Schneider⁽⁸⁾ reported a form of 'snuffles' caused by the rudimentary development and curvature of the turbinated and ethmoid bones leading, in some cases, to a bloody purulent nasal discharge. Iminger,⁽⁹⁾ after observing a large number of cases, decided that the disease was infectious. He reported cases in which the cerebral tissues were involved.

Friedberger and Frohner⁽¹⁰⁾ pointed out that several of the German authors had described a disease of pigs which is evidently not uniform in character, since sometimes the primary ailment seemed to be rickets, and at others a chronic hemorrhagic, suppurative nasal catarrh pos-

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² This disease has been called bull nose, snuffles or necrotic rhinitis by veterinarians and swine owners in the United States.

sibly of an infectious nature. Friedberger and Frohner also classed rhinitis in swine under two forms (a) catarrhal and (b) ricketty. Koske⁽¹¹⁾ attributed an infectious form which he studied, to *Bacillus pyocyaneus*; and, since that time, most writers on the subject have given that organism as the cause of infectious rhinitis, and rachitis as the cause of noninfectious rhinitis. These writers include Hutyra and Marek,⁽¹²⁾ Craig,⁽¹³⁾ and Lynch.⁽¹⁴⁾ Kinsley,⁽¹⁵⁾ however, reviewing the German publications under the title, "Infectious Nasal Catarrh," describes acute and chronic forms of rhinitis, both of which he attributes to the inhalation of dust or other irritating substances, to sudden changes in temperature, or to association with other diseased conditions, particularly swine plague and hog cholera.

In 1917, a leaflet, "Necrobacillosis in Pigs,"⁽¹⁶⁾ was issued by the United States Bureau of Animal Industry in which *necrotic rhinitis* was listed among the pathological conditions considered to be necrobacillosis, and the statement was that "the origin of all forms of necrobacillosis is the *Bacillus necrophorus*." In the following year, Graham⁽¹⁷⁾ described necrotic rhinitis, 'sniffles' or bull nose as being caused by *Bacillus necrophorus*.

Some bacteriological examinations of neoplastic tissue from the sinuses of the head, from the snout and from the scrotal region of swine were made by the writer in 1926-1927 at the School of Veterinary Medicine, State College, Pullman, Washington. In every case, a microscopic examination showed the presence of spirochetes. At the University of California, Branch of the College of Agriculture at Davis, abundant material became available, and here, also, examination of the neoplastic tissue from a large number of pigs always revealed the presence of spirochetes. However, the writer found indications that the spirochetes might be the specific cause of both rhinohyperplasia and scirrhous cord in swine. These organisms, in addition to being always present, were observed close to the line of necrosis and also deeper in the neoplastic tissue than other organisms.

In 1894, Theobald Smith⁽¹⁸⁾ submitted a report entitled "Coarser and Finer Spirilla in the Intestines of Hogs." Twelve years later, Dodd⁽¹⁹⁾ described a disease of the pig due to a spirochete, which he considered the cause of superficial bodily ulcers that averaged about three-fourths of an inch in diameter. These spirochetes varied in length from 9 to 26 microns, the average being from 14 to 16 microns, and with from 2 to 6 spirals. Cleland⁽²⁰⁾ of West Australia published in 1908 an article on spirochetes in castration tumors of pigs. This spirochete was 10 to 24 microns long, with 2 to 6 spirals. King and Baeslack⁽²¹⁾ in 1912, published a report stating that they found spiro-

chetes in the blood of hogs suffering from hog cholera. King, Baeslack, and Hoffman⁽²²⁾ in 1913, observed spirochetes in the blood of hogs infected with cholera. This organism averaged from 5 to 7 microns in length and 1 micron in width. Later in the same year, King and Hoffman⁽²³⁾ published an article entitled "Spirochaeta Suis as a Pathogenic Organism in Hog Cholera." Nomi and Matsuo⁽²⁴⁾ published in 1922 their studies on spirochetes in swine, and in a synopsis of the work in English at the end of the article divided the organisms into two general groups as follows: "One is of a very fine spiral form about 10 to 15 microns in length, and some of the organisms belonging to this group are 27 microns long, but they seem to have developed into a stage of division. The spirals are irregular and vary in number from 2 to 8 or even more. The spirals are 0.5-5 microns long and 1-2 microns deep. The dimensions of the body are too small to allow of measurement, but appear to be about $\frac{1}{4}$ micron. The other is 10-15 microns long and twice as wide as the former and well observed by the use of Giemsa's stain. The spirals are relatively regular and about 10 in number. The length and depth of the spirals are 1 micron." Schmid⁽²⁵⁾ in 1925, described a disease occurring in southwest Africa in which pigs were affected with lesions on the skin and tumor-like swellings on different parts of the body. Microscopic examinations of material from these lesions showed immense numbers of spirochetes. Descazeaux⁽²⁶⁾ in 1926, reported finding spirochetes in affections of pigs in Chile. He was unable to reproduce the affection by intracutaneous or subcutaneous inoculations of healthy pigs.

GROSS APPEARANCE OF THE NEOPLASTIC FORMATIONS AND RHINOHYPERPLASIA

In the cases studied by the writer, the tumor-like formations located in the region of the castration wounds varied from 2 cm to 30 cm in diameter. The weights of these formations after surgical removal varied from 2 ounces to 22½ pounds. In the early stages, the mass was very firm; but later, fluctuating areas were found, upon palpation. Abscess formation took place, later breaking down and forming fistulous tracts to the outside. Foul-smelling fluid was constantly emitted, which dried over the exterior, forming a crust of varying thickness. In these cases, the fluid was enclosed and caused even more intense destruction of tissue.

In the cases of rhinohyperplasia, the face was more or less distorted. There was a severe inflammation of the membranes lining the nose,

and in some cases gangrenous and necrotic areas were found. There was a great thickening of the snout; and, in the more chronic and long-standing cases, necrosis of the bones was not infrequent. Some animals showed bulging of the bones of the face, due to pressure of the accumulating inflammatory material in the sinuses; and this pressure on adjacent structures caused various anatomical alterations. The most common complication was the obstruction of the nostrils which prevented sufficient passage of air.

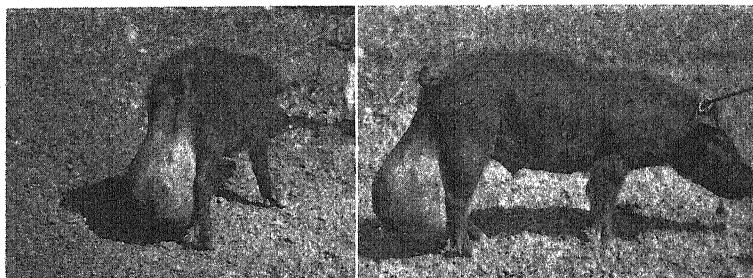


Fig. 1. Tumor-like formations located in the region of the castration wounds, the weight of which after surgical removal was $22\frac{1}{2}$ pounds.

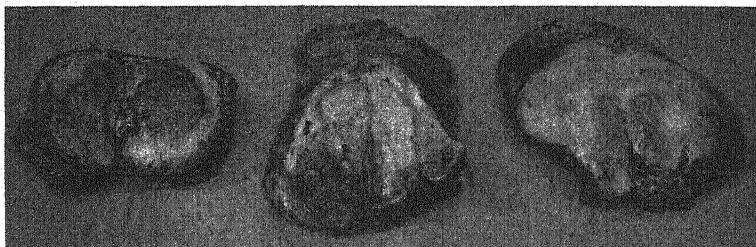


Fig. 2. Scirrhouous cords after surgical removal; opened to show internal aspects of the growths.

The writer, after a careful study of a great number of cases of uncomplicated rhinohyperplasia, has been unable to reveal any gross skeletal changes, such as beading and bending of the ribs, deformity of the sternum, enlargement of the costochondral junctions or epiphyseal ends of the long bones so characteristic of mammalian rickets. Craniotabes were never demonstrable in the flat bones of the skull. Histological evidence of rickets has never been present in any of the cases examined with this condition. At this Station a study of the effect of light rays and restricted nutritional regimes on skeletal deformities of hogs has been carried on by E. H. Hughes of the Animal Husbandry Division. In this work 75 head of hogs have been maintained for

several months in extremely advanced stages of rachitis without any evidences of rhinohyperplasia having been developed. This would seem to have effectively eliminated the supposition that this condition is caused by or constitutes a complication of rachitis in swine, despite the apparent confusion of the two conditions by the German workers cited in the review of literature at the beginning of this paper.



Fig. 3. Rhinohyperplasia showing great thickening of the snout giving the face a distorted appearance.

BACTERIOLOGY

Three distinct organisms were ever present in smears from rhinohyperplasia and scirrhous cord conditions. There were *Bacillus subtilis*, a common saprophyte in chronic suppurative lesions; *Staphylococcus pyogenes albus*, a secondary invader and a frequent cause of abscesses, boils, and surgical suppurations; and lastly, a spirochete. An unidentified Gram-negative bacillus with terminal spores was present in cultures from only two scirrhous cord cases. It grew seemingly in symbiosis with *Bacillus subtilis*, and could not be obtained in pure culture. *Bacillus necrophorus* was isolated from three cases of rhinohyperplasia in all of which the enlargements on the snout had ruptured before the animals were brought to the laboratory. One other animal, pig No. 127, with a scirrhous cord, had an open lesion on the right front leg just above the knee joint. *Bacillus necrophorus* was isolated from this lesion, but the writer was unable to isolate the organism from the scirrhous cord. This animal afterwards developed an infection of the right front foot, which later ruptured; the material from this lesion was found to contain *Bacillus necrophorus*. Spirochetes were found present in the scirrhous cord, but not in either lesion on the leg.

Subcutaneous inoculations were made into rabbits with material obtained from the lesions in six scirrhous cord and rhinohyperplasia cases respectively. In cases where *Bacillus necrophorus* was found, the rabbit usually succumbed in from five to seven days. A second and a third rabbit were inoculated in a similar way, with the exception that the material used was from necrotic areas found in the liver of the first rabbit injected. Following this procedure, the writer was able to obtain the necrophorus organism in practically a pure culture.

Bacillus subtilis and the *Staphylococcus pyogenes albus* were readily isolated in pure culture. The same cannot be said of the spirochete. All attempts to cultivate it anaerobically, on whole blood, blood serum, ascitic agar, and in Hiss serum water, failed. The spirochetes were most successfully demonstrated in direct smears by Giemsa's stain. Almost equally good results were obtained by gentle steaming with carbol-fuchsin for three minutes. The spirchetes exhibited a considerable degree of flexibility, since they straightened out easily in direct smears. They displayed great regularity in their convolutions when stained in the tissue by Levaditi's or by Warthin's methods. In no case did the spirochetes show a tendency for circle formation, V, or Y shapes; neither did they present the right angle bending so characteristic of *Treponema pallida*. The average measurements of the spirochetes were as follows:

<i>Direct smears</i>	<i>In tissue</i>
Length 9 to 10 microns (variations, 6 to 12 microns)	7.37 microns (variations, 4.38 to 10.22 microns)
Number of turns 4 (variations, 3 to 5)	5.88 (variations, 4 to 7)
Spiral depth 0.2 to 0.6 micron	0.86 micron (variations, 0.73 to 1.46 microns)
Amplitude 0.6 to 0.8 micron	
Width 0.1 micron	1.15 microns
	0.24 micron

The spirochete ends were blunt rather than tapering. Motility in contents fresh from the rhinohyperplasia and scirrhous cord, as shown by dark field illumination, was of a rotatory nature.

LOCATION OF THE SPIROCHETES

The spirochetes are found in the tissue just where the line of necrosis and hyperplastic tissue meet. They lie in the minute lymph spaces between the cells and the various fibrils. It has been impossible to demonstrate spirochetes in the blood smears made from the general circulation and obtained under conditions preventing any contamination with spirochetes on the skin or mucous membrane. Smears were

taken from all the animals affected; and, after an extensive search, it was proved conclusively that their presence in the blood stream would be accidental. Microscopical preparations and cultures were made from the liver, spleen, lymphatic glands, kidneys, bile, and urine, but all attempts to locate spirochetes were unsuccessful.

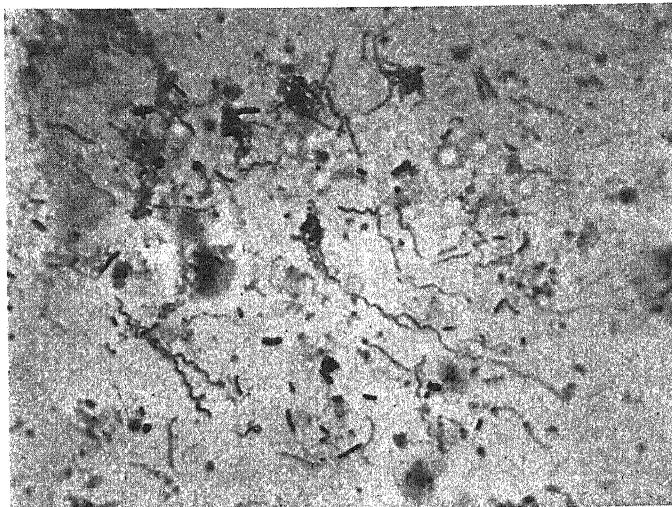


Fig. 4. Spirochetes in smear from scirrhouous cord of pig No. 1. Giemsa's stain. Oil immersion, 2 mm. Ocular No. 15.



Fig. 5. Spirochetes in smear from rhinohyperplasia of pig No. 149. Giemsa's stain. Oil immersion 2 mm. Ocular No. 15.

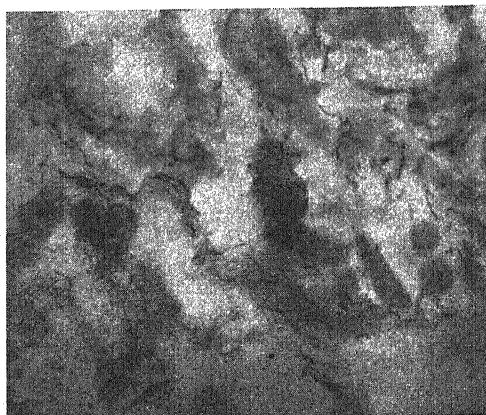


Fig. 6. Spirochetes in tissue lying in the minute lymph spaces between the cells and the various fibrils. Stained by Levaditi's method. Oil immersion 2 mm. Ocular No. 10.

HISTOPATHOLOGY

The neoplasms of inflammatory origin, formed at the end of the cord and tunics, are characterized by a slow proliferative process, due in part to an infiltration and proliferation of the fibroblasts, often combined with lymphocytes, polymorphonuclear leukocytes, and endothelial cells. The peripheral portion of the tumorous mass is made up of a dense, fibrous connective tissue composed of spindle or star-shaped fibroblasts, with a considerable variation in the size and shape of the nuclei of the cells, and occasionally with mitotic figures present. The stroma contains collagen and elastic fibers and is edematous in places. There are a large number of arborized blood vessels throughout the mass. Close to the line of demarcation between the hyperplastic tissue and the moist gangrenous area, there is an alteration in the blood vessel walls, and some may be seen plugged with thrombi and leukocytes. Some of the small veins lying in the area close to the line of necrosis show thrombosis; others exhibit areas of localized leukocytic infiltration involving the area between the intima and media, so that the intima is raised and thrown into convolutions until in places its continuity is even broken.

At the line of demarcation between the hyperplastic tissue and the gangrenous area, there is a zone composed of a great number of lymphocytes, polymorphonuclear leukocytes, some fibroblasts, and great numbers of spirochetes. As this zone reaches the gangrenous area, the cells become degenerated, swollen, and granular; the nuclei

exhibit karyorrhexis, karyolysis, and pycnosis; and many cells have completely disintegrated. Droplets of fat, bacteria, and cell detritus make up the remaining gangrenous area.

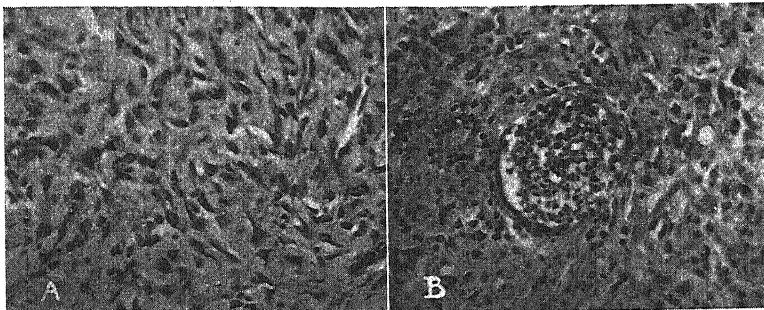


Fig. 7. A, high power view of neoplastic tissue made up of dense fibrous connective tissue, having spindle and star-shaped fibroblasts, collagen and elastic fibers. B, close to the line of demarcation between the hyperplastic tissue and the moist gangrenous area, the blood vessels may be seen plugged with thrombi and leukocytes. High power view.

In rhinohyperplasia, the chronic inflammation of the nares lacks the histological features of a new growth, and in fact many cases showed nothing more than localized edematous areas on the mucous membranes. Over the snout there is a progressive hyperplasia of fibrous connective tissue. In long-standing cases, there is a pronounced

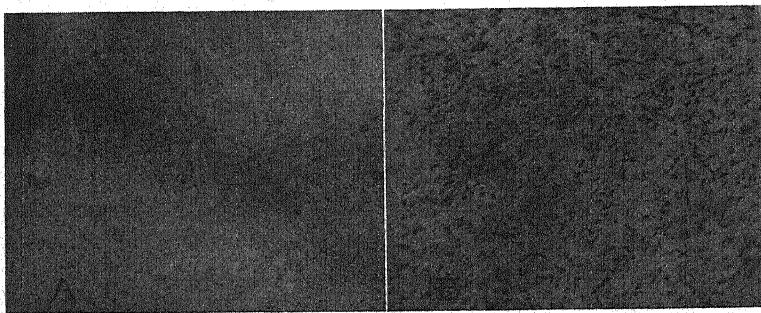


Fig. 8. A, low power view, showing line of demarcation between the necrotic area and the hyperplastic tissue. B, high power view of hyperplastic connective tissue.

inflammation of the periosteal coverings of the bones, later resulting in necrosis and sequestra. Where the sinuses are involved, there is an inflammation and thickening of the mucosa, with an accumulation of necrotic, foul-smelling material, which causes a bulging of the already affected bones.

Throughout the histological examination of all the tumorous masses derived either from scirrhoue cord or from rhinohyperplasia, the structure has remained remarkably uniform. There is always that sharp line of demarcation between the gangrenous or necrotic area and the surrounding hyperplastic connective tissue, where the spirochetes are found lying in the minute lymph spaces between the cells and the various fibrils.

ANIMAL INOCULATIONS

Pigs 45 and 46, Duroc Jersey boars, weighing 40 pounds each, in good thrifty condition, were inoculated on August 30, 1928, with 0.1 cc of material containing spirochetes taken from a necrotic area of scirrhoue cord from pig No. 1. This infective material was placed into the castration wounds after removal of the testicles. These animals developed scirrhoue cords which measured approximately 22-23 cm in diameter on December 4, 1928. Microscopic examination of material discharged from this growth showed the presence of spirochetes.

Pig No. 47, a Poland China boar, weighing 45 pounds, in good thrifty condition, was inoculated on September 4, 1928, with 0.1 cc of material containing spirochetes taken from scirrhoue cord of pig No. 3. The infective material was placed into a pocket-like incision on the snout, and 1 cc of the same material was put into the castration wound after removal of the testicles. This animal developed a scirrhoue cord and typical rhinohyperplasia. Spirochetes were found present in the necrotic material from both places.

Pigs 11 and 12, both Poland boars, weighing 40 pounds each, were inoculated on September 4, 1928, with 1 cc of material containing spirochetes from scirrhoue cord of pig No. 3. Material was placed into an abrasion in the nasal passages. The animals developed enlargements approximately 4 cm in diameter that later ruptured and emitted a foul-smelling material which, on examination, showed the presence of spirochetes. On September 20, 1928, both of these animals showed a characteristic rhinohyperplastic condition. The enlargement was incised, the foul-smelling necrotic material was removed, and powdered antimony and potassium tartrate was placed in the cavity. Four days later, a considerable decrease in the size of the swelling was noticed. On December 4, these animals showed very little deformity of the face and had apparently recovered from the infection, the hogs weighing approximately 150 pounds each.

Pigs Nos. 17 and 18, Duroc Jersey boars, weighing about 35 pounds each, and in good healthy condition, were inoculated with 0.1 cc of material containing spirochetes taken from necrotic material of pig No. 45 on November 23, 1928. This material was placed into wounds made on the snout. The animals developed characteristic lesions of rhinohyperplasia.

Pig No. 19, a Poland China sow, weighing approximately 25 pounds, in good healthy condition, was inoculated with 0.1 cc of necrotic material containing spirochetes taken from scirrhous cord of pig No. 46. The material was placed in a pocket-like incision in the subcutaneous tissues of the shoulder. Three days later this animal showed characteristic gangrenous lesions, such as is found in scirrhous cord and rhinohyperplasia. There was a discharge of foul-smelling material from the wound, which, upon examination, showed the presence of spirochetes. This gangrenous area continued to enlarge and on December 4 was 4½ cm in diameter.

Pigs 63, 64, and 65, Duroc Jerseys, weighing approximately 30 pounds each and in good health, were inoculated on December 25, 1928, with 0.2 cc of material containing spirochetes obtained from a scirrhous cord and rhinohyperplasia formation. The inoculations were made subcutaneously over the shoulder. On December 30, 1928, swellings about 4 cm in diameter had developed. These swellings finally ruptured, discharging a foul-smelling material which contained great numbers of spirochetes. At the point of inoculation, the lesions increased in size; and on January 10, 1929, they were 9 cm in diameter; the openings to the exterior were 5 cm in diameter. The crater-like lesions were gangrenous, necrotic, and macroscopically comparable to the internal aspect of rhinohyperplasia or scirrhous cord.

Pigs 61 and 62, Duroc Jerseys, weighing approximately 38 pounds each, in good healthy condition, were inoculated on December 25, 1928, with a small piece of tissue removed from a scirrhous cord. This inoculation was made into a pocket-like incision over the shoulder and into the snout. This piece of tissue was taken from the hyperplastic area of scirrhous cord close to the line of necrosis. A portion of this tissue was imbedded, sectioned, and stained; on microscopical examination it was found to contain no organism other than spirochetes. These animals developed characteristic gangrenous lesions, such as are found in scirrhous cord and rhinohyperplasia. Necrotic material taken from these lesions showed, on microscopical examination, great numbers of spirochetes.

Pigs 63 and 64, Poland China, weighing approximately 25 pounds each in good healthy condition, were inoculated on May 7, 1929, with a

pure culture of *Bacillus necrophorus* into two pocket-like incisions over the shoulders. They developed small abscesses 1 to 2 cm in diameter at the point of inoculation, which later ruptured exuding a small amount of thick tenacious cheesy pus. The lesions were in no way similar to those produced by the inoculation of tissue containing spirochetes. On May 27 a necropsy showed no internal lesions, and spontaneous healing had taken place at the point of inoculation, leaving a small amount of scar tissue.

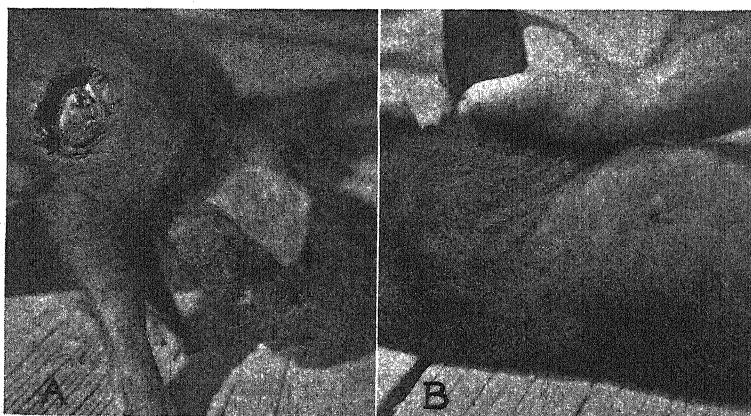


Fig. 9. A, pig No. 61, inoculated subcutaneously over the shoulder and snout with small piece of tissue from scirrhus cord containing spirochetes. B, pig No. 63 inoculated with a pure culture of *Bacillus necrophorus*; spontaneous healing took place at the point of inoculation, leaving a small amount of scar tissue.

A pure culture of *Bacillus necrophorus* not being obtainable from the "American Type Culture Collection," it was necessary to isolate a pure culture here at this Station. This was done by the following procedure. Necrotic material was taken from the mouth of a calf affected with calf diphtheria, which on microscopic examination contained great numbers of necrophorus organisms. This infectious material was placed into pocket-like incisions on the side of two rabbits, which succumbed in 4 and 6 days, respectively. These animals showed abscesses in the liver, and material taken from these lesions was inoculated into a second series of rabbits, which on autopsy showed abscesses in the liver and kidneys. This procedure was carried on through a third and fourth series of rabbits. Infective material derived from the fourth series of animals was inoculated into gelatin agar media and placed in hydrogen jars at a temperature of 37.5° C. Great numbers of small gas bubbles appeared, followed by small yellowish-white colonies which developed in seventy-two hours.

INOCULATION WITH PURE CULTURES OF ORGANISMS
OTHER THAN SPIROCHETES, CULTIVATED FROM
SCIRRHOUS CORD AND RHINOHYPERPLASIA

Pig No. 20, a Duroc Jersey boar, weighing 35 pounds, in good healthy condition on November 23, 1928, was thoroughly washed to prevent any contamination of the skin with any spirochetal infection and was placed in a lot that had never contained hogs affected with scirrhouus cord or rhinohyperplasia. The animal was castrated and 1 cc of a pure culture of *Staphylococcus pyogenes albus* was inoculated into the wound after the removal of the testicles.

On November 25, a slight swelling was noticed at the seat of castration, which afterwards developed a small abscess; this ruptured, and there was a continual discharge from the wound for several days. The discharge was examined microscopically and found to be free from any spirochetes. This castration wound healed up completely on December 4, with a very slight thickening of the cord and skin at the seat of castration.

Pig No. 21, Duroc Jersey boar, weighing 32 pounds, in good healthy condition, was prepared in the same manner and placed in the same enclosure with pig No. 20. The animal was castrated; 1 cc of a pure culture of *Bacillus subtilis* was inoculated into the wound after removal of the testicles. On November 25, there was a discharge from the wound and some thickening at the margin of the incision. This animal failed to develop a scirrhouus cord, and on December 4 appeared perfectly normal.

Pig No. 22, a Duroc Jersey boar, weighing 37 pounds, in good healthy condition, was prepared in like manner, castrated, and inoculated with 1 cc of mixed culture of a Gram-negative bacillus and *Bacillus subtilis* into the wound after the testicles had been removed. On November 25, there was some thickening of the wound and a discharge from the incisions. This animal also failed to develop a scirrhouus cord and appeared perfectly normal on December 4.

Pig No. 23, a Poland China boar, weighing 37 pounds, in good healthy condition was prepared in the same manner, castrated, and inoculated with 2 cc of a pooled culture of these organisms, including the *Staphylococcus pyogenes albus*, *Bacillus subtilis*, and a Gram-negative bacillus, into the castration wound after removal of the testicles. On November 25, this animal had considerable discharge

from the castration wounds. There was some thickening and induration of tissue in the locality of the operation. On December 4, this animal had failed to develop a scirrhous cord, and appeared normal with the exception of a small amount of thickening at the seat of castration. Pig 20, 21 and 23, December 26, were sold as healthy animals and free of scirrhous cord.

ATTEMPTS TO TRANSMIT THE SPIROCHETES TO ANIMALS OTHER THAN THE PIG

On August 30, 1928, four guinea pigs (Nos. 1, 2, 3, and 4) weighing approximately 350 grams, in good healthy condition, were castrated; 0.1 cc of necrotic material, containing spirochetes taken from pig No. 1, was inoculated into the wound after removal of the testicles. These animals developed, at the seat of inoculation, abscesses which afterwards were incised; material taken from them was examined microscopically. It was found to contain *Staphylococcus pyogenes albus*, *Bacillus subtilis* and other contaminating organisms, but was negative for spirochetes. These animals failed to produce any gangrenous lesions like those found in the hog, and after repeated inoculations of other guinea pigs, it was impossible to produce a spirochetal infection.

Two buck rabbits (Nos. 99 and 17) in good healthy condition on September 4, 1928, were castrated and 0.1 cc of necrotic material from scirrhous cord of pig No. 3, containing spirochetes, was inoculated into the wound after removal of the testicles. Abscess formation developed. After the lancing of these enlargements, the material was microscopically examined and found negative for spirochetes. Subcutaneous inoculations of 0.1 cc of material containing spirochetes were made into the same animals on September 20. These inoculations also failed to produce typical gangrenous lesions like those found in the pig; and upon microscopic examination, the material from these wounds was found negative for spirochetes and also for *Bacillus necrophorus*.

THERAPEUTIC AGENTS IN THE TREATMENT OF SCIRRHOUS CORD AND RHINOHYPERPLASIA

Therapeutic agents were tried in combating the spirochetes. It was believed to be necessary to use a drug having some specific action on this type of organism. As the infecting agent was not found inhabiting the blood stream, intravenous injections were not tried. Because of the location of the spirochetes, it was necessary also to have a drug which would penetrate into the tissue enough to attack the organism. Antimony and potassium tartrate (tartar emetic) seemed to have the desired qualities. Most arsenical preparations such as salversan, etc., used in human spirochetosis, would be too expensive.

The tumor-like growths were incised to allow free drainage, the necrotic, foul-smelling contents were removed, and powdered antimony and potassium tartrate was placed into the cavities. Enough of the drug should be used to coat over the inside area affected. If too much of the powder is placed into the cavity in the presence of a thin, fibrous capsule, absorption will be so great as to produce poisoning and death.

Antimony and potassium tartrate was used in an aqueous solution and in glycerin, but the results were unsatisfactory.

Sodium cacodylate was tried with varying results; more experimental work will be necessary to prove its value as a therapeutic agent in these conditions.

Animals Treated with Antimony and Potassium Tartrate.—Pigs Nos. 11 and 12, both Poland China boars, weighing 40 pounds were inoculated on September 4, 1928, with 1 cc of material containing spirochetes from scirrhouus cord of Pig No. 3. Material was placed into an abrasion in the nasal passages. The animals developed an enlargement approximately 4 cm in diameter that later ruptured and emitted a foul-smelling material, which on examination showed the presence of spirochetes. On September 20, 1928, both of these animals showed a characteristic rhinohyperplastic condition. The enlargement was incised, the foul-smelling necrotic material was removed, and powdered antimony and potassium tartrate was placed in the cavity. Four days later a considerable decrease in the size of the swelling was noticed; and on December 4, these animals showed very little deformity of the face and had apparently recovered from the infection, the pigs weighing about 150 pounds each.

Pigs 61 and 62, both Poland China gilts weighing 65 pounds, had rhinohyperplasia. On December 12, 1928 the enlargements were incised, necrotic material was removed, and powdered antimony and potassium tartrate was placed in the cavities. The enlargements receded and the wounds healed, leaving just a small thickening over one side of the snout. The animals were discharged as cured on December 26.

Pigs 100 to 138, 38 animals in all, Duroc Jerseys, Poland Chinas, and Berkshires, had scirrhouss cords approximately 2 to 6 cm in diameter, these growths having developed within the two weeks after castration. On December 21, 1928, the growths were incised, and after the necrotic material had been removed from the center, enough powdered antimony and potassium tartrate was then placed into the cavities to coat over the surface. There was considerable swelling within the next 12 hours, followed by an increase in the discharge from the wounds. One week later, the animals were examined, any loose necrotic material remaining was removed, and the cavities were washed with a mild antiseptic solution. Fourteen days following the placing of the powdered antimony and potassium tartrate in the cavities of these seirrhous cords, the animals were discharged as cured and sold on January 9, 1929.

SUMMARY

Spirochetes were present in all tissue specimens of scirrhouss cord and rhinohyperplasia. The spirochetes were easily demonstrated in the discharge from these conditions when stained with carbol-fuchsin and Giemsa's stains. In the tissue preparations, stained by Levaditi's and Warthin's methods, the spirochetes were observed close to the line of necrosis in the hyperplastic tissue.

Bacillus subtilis and *Staphylococcus pyogenes albus* were also always present in the discharge, and a Gram-negative bacillus was found occasionally, which, when cultured, seemed to grow in symbiosis with *Bacillus subtilis*.

Bacillus necrophorus was isolated from three cases of rhinohyperplasia, and the organisms were associated in each case with spirochetes. As a result of the observations and experimental work, the conclusion seems justified that the presence of spirochetes in every case indicates that they are the etiological factor in these hyperplastic growths, and the *Bacillus necrophorus*, a secondary invader.

The spirochete has not been cultivated in pure culture. The spirochetes are located more deeply in the lesions than the other organisms, this fact suggesting their pathogenic influence, and etiological relationship in the production of scirrhous cord and rhinohyperplasia.

Scirrhous cord and rhinohyperplasia could not be experimentally reproduced by injection of the pure cultures or pooled cultures of organisms other than spirochetes isolated from these lesions. When, however, the mixed cultures containing the spirochetes were injected, the diseases were readily reproduced.

The inoculations of pure cultures of *Bacillus necrophorus* into healthy pigs, did not produce lesions similar to those obtained by the inoculation of tissue containing spirochetes.

Work done heretofore on the effect of light rays and restricted nutritional regimes on skeletal deformities of hogs has effectively eliminated the supposition that rhinohyperplasia is caused by or constitutes a complication of rachitis in swine.

Antimony and potassium tartrate (tartar emetic) was tried as a therapeutic agent, with about 90 per cent recoveries; but precautions were necessary to prevent poisoning from absorption because of too large amounts placed in the incised growths.

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STUDIES OF THE BREEDING OF SUGAR BEETS FOR RESISTANCE TO CURLY TOP

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INTRODUCTION

The nature of the injury caused by curly top and the importance of this disease to the sugar-beet industry in certain western states have been recognized for many years and have been discussed by Ball,⁽²⁾ Carsner and Stahl,⁽⁵⁾ and Severin.^(9, 10)

Studies of the behavior of beets affected with curly top have shown that individual beets vary as to their susceptibility to this disease. Reference is made here not only to observations of the writer but to those of Carsner and Stahl⁽⁵⁾ and Carsner.⁽⁸⁾ These observations have suggested the possibility of developing curly-top-resistant strains by selecting the least affected individuals among infected beets in commercial fields.

Carsner⁽⁸⁾ has shown that resistance to curly top is an inherent characteristic in individual beets. Several of the strains which he selected for resistance to curly top were definitely more resistant than the commercial beets with which they were compared. Furthermore, he found that some other morphologically uniform strains which were developed without reference to curly top showed constant differences in susceptibility to this disease.

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Some conclusive results with regard to resistance of sugar beets to curly top have also been obtained by the writer. The work upon which these results are based was started by the Spreckels Sugar Company in the Salinas Valley, and was later transferred to the Branch of the College of Agriculture, Davis, California. Several strains resistant to the disease have been developed and their characteristics studied during three years at King City, California, and during two more years at Davis. This work is reported in this paper.

METHOD OF BREEDING FOR RESISTANCE

Curly-top-resistant strains were developed by selection. Plants which showed resistance in commercial fields, in which the beets were severely affected by curly top, served as the initial selections. The appearance of the leaves was employed as the basis of the first selection, which was usually undertaken several weeks before harvest. However, the plants which were seemingly less affected by the disease showed in every case symptoms of curly top; that is, immunity was not observed. At harvest time the previously marked beets were selected for size and shape of root.

The progenies of these mother beets were tested under exposure to infection, and selections were made again within those progenies that showed the highest degree of resistance. In addition to a selection for size and shape, the percentage of sugar of individual roots was determined, and those of low quality were eliminated.

The seed from each mother beet was kept separate and was planted in individual rows. A commercial strain of sugar beet served as a check. The commercial variety known as 'Old Type,' which is produced by the German firm, Rabbethge and Giesecke, was used for this purpose in every case, with the exception of the King City planting of 1925, when a commercial strain produced by the French firm, Vilmorin and Company, was used as a check.

Seed-bed conditions made necessary the sowing of seed at a heavy rate and by machine. Consequently, the amount of seed from individual mother beets was not sufficient to make more than two or three replications of each progeny. The commercial seed which served as a check was planted in many replications. The usual practice was to sow four progenies in four individual rows 50 or 100 feet long, and the fifth row with commercial seed for a check. Whenever possible the sets of five rows were repeated two or three times. This method was somewhat modified in 1929 when each progeny was sown in four rows 25 feet long, and the fifth and sixth rows were employed as a check.

The preparation of the seed bed was similar to that of a commercial beet field. Manure and commercial fertilizers were not applied in the King City experiments. The Davis tests were conducted upon a field which had been manured repeatedly in the past. The irrigation water was applied at two-week intervals, and the cultivation was done with wheel hoes.

The harvesting was done by hand. The total weights were determined in the field. Since some of the beets had to be saved for propagation, the topping was not done in the usual manner, but the leaves were cut off about one inch above the crown. With the exception of the 1929 test, this procedure was followed throughout. In 1929 the beets were topped in a manner followed in commercial practice.

The methods of propagating the seed will be discussed in connection with the description of the resistant strains.

METHODS AND CONDITIONS OF TESTING FOR RESISTANCE

Resistance Tests in the Salinas Valley.—According to Severin and Henderson,⁽¹¹⁾ the interior regions of the Salinas Valley are within the boundary of the natural breeding areas of the beet leafhopper (*Eutettix tenellus* Baker). They have been subject to epidemics of curly top almost every year since the beginning of beet culture in these regions, and the disease appeared consistently in a form which Carsner⁽¹⁴⁾ designated as severe. For this reason the King City and the Greenfield districts, which are located in the interior of the Salinas Valley, were selected for the resistance trials. In order to assure a severe natural infection it was only necessary to plant the seed late in the spring, so as to have the beets at a very young stage at the time of appearance of the beet leafhopper. Carsner and Stahl⁽¹⁵⁾ have shown that beets are most susceptible to curly top in their early stages.

During the three seasons when extensive trials were conducted in the Salinas Valley, the conditions for infection with curly top were favorable. In 1925 the beets at Greenfield were sown on March 13, eleven days before the influx of leafhoppers, and at King City on March 24, after leafhoppers had appeared in the beet fields. As a rule, the conditions with regard to curly top at Greenfield are very similar to those at King City, but in 1925 the plot at Greenfield was exposed to a more severe infection than the other. The beets at Greenfield were up when the leafhoppers appeared. Before they reached the thinning stage the commercial beets adjoining the trial field were

plowed under, and the leafhoppers accumulated upon the experimental plot. At King City, on the other hand, no exceptionally large accumulations of insects were observed upon the test plot; the beets came up after the first appearance of the leafhoppers, and the adjoining beet fields were not plowed under. Also, with regard to cultural and soil conditions, the King City plot was far better placed. Consequently, although at the beginning of June every plant in the check rows in both plots was showing unmistakable symptoms of curly top, the effect of the disease upon the King City beets was not so detrimental as upon those in the plot at Greenfield. The beets on both plots were harvested in the middle of November.

In 1926 the beets were planted on March 29 and 30 at King City. The first influx of leafhoppers occurred on April 19, when the beets were still too small for thinning. As the trial plot was the only planting of beets in that section of the farm (the nearest commercial beet field was about six miles away), the insects during the spring flights apparently congregated upon this plot. A count of the number of leafhoppers taken at this time showed a population of 20 to 30 insects to 100 feet of row. Because of warm weather at the time of influx, symptoms of the curly-top disease developed very rapidly, and on May 15 all of the beets in the check rows were diseased. The beets were harvested in the latter part of October.

In 1927 the plantings were made at King City at two different dates—one on April 4, before the influx of leafhoppers, the other on May 5, after the influx. During the entire season the two plantings showed a considerable difference in the general development of the beets. The earlier planting was exposed not only to a very severe infection with curly top, but also to other unfavorable growth conditions. In the first stages of germination the young seedlings were weakened through the formation of a crust on the surface of soil after a rain, and they developed only slowly on account of the cold weather, which persisted until the latter part of April. About April 21 a sudden rise of temperature occurred, and great numbers of aphids and leafhoppers appeared in the beet plots. The number of leafhoppers was found to be 30 per 100 feet of row. In the latter part of May all of the commercial beets showed symptoms of the disease. As to the beets that were planted later, the weather was moderately warm during germination of the seed, and the seedlings were not weakened by crust formation or by aphid injury. Throughout the season the May-planted beets had a healthier appearance than those planted in April, although by the middle of June the percentage of diseased beets was the same in both plots. Thus, as in 1925, the beets that were up at the

time of influx were injured far more than those that were sown after the leafhoppers had appeared. The April planting was harvested on October 17 and 18, and the May planting on October 27.

Resistance Tests at Davis.—In the fall of 1927 the work on resistance was transferred to the Branch of the College of Agriculture, Davis, California. The conditions here were very different so far as concerns natural infestation by the leafhopper.

According to Severin and Henderson⁽¹¹⁾ the Davis district is located in a migratory area of the leafhopper. Observations of the past have shown that the outbreak of the disease can be expected here with less certainty than in the Salinas Valley, especially in years of decreased population of *Eutettix tenellus*. In fact, no influx of leafhoppers was observed in 1928 and 1929, and the insects had to be introduced artificially. A stock of viruliferous leafhoppers was reared in a greenhouse upon potted sugar beet plants in insect cages of the type shown by Severin.⁽⁹⁾ In the spring the insects were released upon the young beets in the trial field.

During the winter of 1927-28 about 6000 leafhoppers were reared, and the beets were inoculated by dropping three to four viruliferous nymphs between the heart leaves of each plant. The beets were sown on March 17 and were up on March 24; and the nymphs were added on May 15. Exceptionally favorable weather conditions were responsible for quick germination of the seed and for the rapid growth of plants. Consequently, when the leafhoppers were released (May 15) the beets were far advanced in their development and were only slightly injured by the disease. On June 10, the check rows showed 100 per cent infection, and on June 26 they could readily be distinguished from the rows of resistant beets by the yellow color of the older leaves and by the severe symptoms, such as distorted veins and warty protuberances, upon the younger. The reduction in yield, however, was very much less than in other years. The beets were harvested by the middle of October.

In 1929 the conditions for testing for resistance were far more favorable than in 1928. It was possible to rear a larger population of insects and have them available at an earlier date than in the previous season. The beets were planted on March 15 and 21; they were up on March 25 and 31, and the insects were released on April 23 and 27, respectively. Approximately 15,000 adult leafhoppers were released upon an area of 20,000 square feet. This time the insects were not placed upon individual beets, but were distributed over the whole area as uniformly as possible by being shaken out of the cages along the rows of beets.

The beets, which were thinned a week before the date when the insects were released, had four foliage leaves on the day of inoculation. The warm, dry weather of the following week was very favorable for the development of curly top. The first symptoms appeared on May 1. A few days after the first irrigation, given on May 14, all the beets in the check rows showed symptoms of the disease with the exception of a few individuals which evidently escaped the inoculation at the beginning. On May 21 the first nymphs of the new brood were observed. The disease continued to spread, and on July 17 all the beets in the check rows were infected. The beets were harvested during the first part of October.

DEVELOPMENT AND TESTS OF THE P19 STRAIN

The breeding experiments with sugar beets with regard to resistance to curly top were started by the Spreckels Sugar Company in 1919,² during a year of a disastrous outbreak of the disease. The extent of injury caused by curly top in 1919 may be well demonstrated by the low yield of sugar beets at King City, which amounted to only 1.4 tons per acre as an average from 2600 acres. Carsner and Stahl⁽⁵⁾ also showed that the yield of sugar beets in several districts of California reached one of its lowest limits in 1919, owing to losses caused by curly top. The severely infected fields offered excellent opportunities for the initial selection of beets for resistance to the disease.

Origin of the P19 Strain.—Five thousand apparently resistant beets were selected in the fall of 1919 in diseased commercial fields at King City. The following season these roots were transplanted for seed production. During the period of blooming the seed beets were covered with hoods of unbleached muslin. Although beet pollen can pass through this type of cloth, it offered considerable protection against cross-pollination, since all the beets that went to seed were covered.

In 1920, 183 of the five thousand beets developed seed stalks. The progenies of these mothers were tested for resistance in 1921. Only one of these progenies was found to exhibit marked resistance to curly top. This one also showed considerable uniformity of morphological characters.

² The data covering the work before 1924 were obtained from the unpublished reports of the Spreckels Sugar Company's Experiment Station at Spreckels, California. Messrs. W. J. Hartung, E. A. Schwing, G. T. Scott, A. A. Tavernetti, and W. W. Thomas of that Station made the first selections for resistance. From 1924 the writer was in charge of the work.

The beets of this resistant progeny were planted for seed production as a group at Spreckels, California. They were not covered during bloom but were sufficiently removed from seed beets of other origin to prevent contamination from foreign pollen. Five mother beets of this group yielded seed in 1922.

All five progenies from these mothers showed resistance to curly top in 1923; one progeny, consisting of seven beets, was conspicuously less affected than others, and it was saved for further propagation. This one progeny yielded in later years a comparatively uniform and markedly resistant strain which has been designated as the 'P19,' the 'P' standing for the parental generation and the '19' for the year of selection of the parent root.

Propagation of the P19 Strain.—From 1925 to 1929 the P19 strain was subjected to five successive trials for resistance. Seed for the third filial generation was obtained from the seven mother beets of 1923. These mother beets were not hooded during blooming but were isolated by distance in groups and individually. Mothers 1, 4, and 7 were left as a group at King City; 2 and 3 were planted together at Spreckels; 5 and 6 were isolated individually at other points in the Salinas Valley. The respective distances between the groups were as follows: between group 2-3 and mother 5, 14 miles; between mothers 5 and 6, 30 miles; between mother 6 and group 1-4-7, about five miles. The seed of these mother beets was tested in 1925. Mothers 1, 2, 3, 4, and 7 gave progenies that were very similar morphologically and markedly resistant to curly top; 5 failed to set seed; and 6 gave a progeny which was far less resistant than the other P19 progenies, and which also deviated from the latter in its morphological characters. No further selections were made from progeny 6.

For the purpose of gaining time in the development of the P19 strain some seed from five (mothers 1, 2, 3, 4, and 7) of the 1923 mother beets was planted in August, 1924, immediately after harvest. No selection was made either for resistance or sugar content. The roots were transplanted in February, 1925, and were exposed to cross-pollination within the group. Twenty-three progenies of the fourth generation were obtained for the trial of 1926.

The test for resistance of 1927 included 100 progenies of the fourth generation from beets selected from progenies 1, 2, 3, 4, and 7 in 1925. For seed production these mother beets were segregated into three groups, according to their size and sugar percentage. There was free cross-pollination within the groups, but the distances between the

groups were 10 and 15 miles. Beets which were isolated individually failed to set seed. The three groups of progenies which were derived from the three groups of mother beets did not show any significant differences from one another.

The beets selected from the P19 strain in 1926 and 1927 gave the progenies of the fifth generation, some of which were still kept separate and were tested for resistance in 1928 and 1929. A few progenies of the fourth generation were tested again in 1929.

TABLE 1

RESULTS OF THE RESISTANCE TESTS OF THE P19 STRAIN (1925 TO 1929)

Location of test plots	Date of seeding	Strain	Genera-tion of P19	Num-ber of P19 proge-nies	Number of beets per 100 feet of row		Percent-age of beets that survived	Roots per 100 ft. of row, kgm.	Average weight per beet, grams
					Early in season	At har-vest			
Greenfield....	March 13, 1925	P19.....	Third....	5	39			11.25	289
		Check.....			81			7.94	98
King City....	March 24, 1925	P19.....	Third....	5	52			30.76	592
		Check.....			86			21.45	249
King City....	March 29, 1926	P19.....	Fourth....	19	49			22.73	464
		Check.....			17			1.97	116
King City....	April 4, 1927	P19.....	Fourth....	100	28	25	89	8.57	342
		Check.....			74	37	49	3.78	102
King City....	April 4, 1927	P19.....	Fourth....	44	29	26	90	8.90	342
		Check.....			71	35	49	3.57	102
King City....	May 5, 1927	P19.....	Fourth....	44	55	54	99	24.41	452
		Check.....			130	80	62	13.55	169
Davis.....	March 17, 1928	P19.....	Fifth....	6	102	99	97	67.98	687
		Check.....			107	101	94	66.92	663
Davis.....	March 15, 1929	P19.....	Fifth....	9	76	69	91	24.25	351
		Check.....			138	49	36	6.47	132
Davis.....	March 15, 1929	P19.....	Fourth....	3	119	112	94	26.87	240
		Check.....			143	87	47	10.09	151

Resistance Tests of the P19 Strain.—Table 1 presents the results obtained with the P19 strain during five successive seasons. The pairs of data represent the averages of the P19 progenies and of their corresponding check rows. The P19 progenies were grouped according to generations and to dates of seeding. Of the two pairs of data which are given for the April planting of 1927, one presents the average of all the hundred progenies of the fourth generation, and the other the average of those forty-four progenies of the fourth generation which were also planted in May of the same year.

In 1925 and 1926 the number of beets was determined only at harvest time; in 1927, 1928, and 1929 the beets were also counted at

the beginning of the season, and the percentage of beets which survived during the test was calculated from these data. It is necessary to point out here that the P19 strain usually gave inferior seed germination, so that the rows of P19 required very little thinning. The small number of beets harvested per row in the P19 resulted, as a rule, from the low viability of the seed. A better stand was obtained with P19 in the Davis plantings, owing to the heavier rate of seeding and better condition of the seed bed; in addition, the seasonal conditions in 1928 were exceptionally favorable for germination. In the

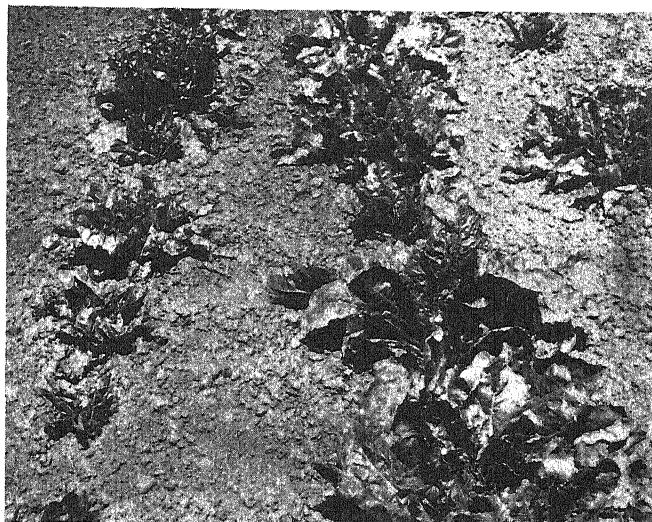


Fig. 1. The effect of curly top upon the foliage of the P19 resistant strain (right) and of a commercial susceptible strain (left). King City, June 18, 1925.

commercial seed the germination was generally satisfactory every season, and the low numbers of harvested beets were due to losses from disease.

It will be noted from table 1 that regardless of the unsatisfactory stand, the P19 strain gave a very much higher yield than the commercial check in every trial except in 1928, when the beets were less affected by curly top.

In figures 1 and 2, two progenies of the P19 strain are shown in comparison with their corresponding checks. Figure 1 shows the effect of curly top upon the foliage of resistant and susceptible beets, and figure 2 upon the yield of roots. In both cases the stand of beets in the P19 row was comparable at the beginning of the season with that in the check row.

Characteristics of the P19 Strain.—The tests over a series of years have shown that the P19 strain is decidedly resistant to curly top and gives a considerably higher yield than a commercial strain when both strains are exposed to a severe infection of the disease. The percentage of beets which survived the infection was consistently very much higher in the P19 strain, and this strain yielded roots of larger average size than the commercial seed.

Immunity, however, was never observed in this strain; all P19 beets were infected at the end of the season, although as a rule they

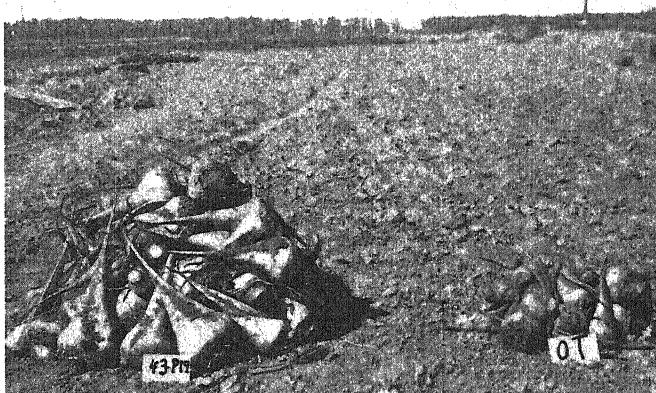


Fig. 2. The effect of curly top upon the yield of roots of the P19 resistant strain (left) and of a commercial susceptible strain (right). The respective yields per 100 feet of row were 41.73 kgm, and 7.40 kgm. King City, October 17, 1927.

developed only the transparent venation, and this symptom was discernible only with some difficulty. The average size of roots was smaller and the percentage of surviving beets was lower when the tests were more severe, a fact which also indicates that the resistance of the P19 strain does not constitute immunity.

The P19 progenies of the third, the fourth, and the fifth generations showed marked uniformity with regard to the tendency to develop only faintly discernible transparent venation and to yield a high percentage of surviving beets. The repeated selections for resistance within the strain, followed by a grouping of the closely related mother beets for seed production, did not increase the resistance of the strain.

There was also a comparatively small range of variation in the type of top and root. In general the P19 strain shows low vigor. The foliage appears light green in comparison with that of other beets. The tops are sparse, and the leaves form a flat, spreading rosette. The roots are, on the average, short and broad, often semi-globular (fig. 3). They show a greater tendency to develop a split crown than other beets grown under similar conditions. As this splitting frequently occurs at an early stage, multiple crowns are a common characteristic of larger P19 beets. There is usually a tinge of pink on the epicotyl, which frequently spreads upon the hypocotyl and the petioles of the leaves.

The greatest differences between the individual progenies of the P19 strain were found in the viability of the seed. A significant variation was also observed in the average size of the plants, which was correlated not with the differences in the stands of beets but with variation in vigor and degree of resistance in the individual progenies.

Sugar Analyses of the P19 Strain.—Determinations of the sugar content were made in 1925, 1926, and 1927, for the purpose of selection of mother beets for further propagation. Only roots of the best shape and size were chosen for the sugar test, and each root was analyzed individually. In 1927, 1928, and 1929, samples of beets of some of the P19 progenies and of their corresponding check rows were also tested as composite samples like ordinary field samples.

The sugar percentage was determined by a method of simple polarization, the Herzfeld modification of the Sachs-Le-Docte process being employed. At Spreckels the process was carried out by hot-water digestion, and at Davis by cold-water digestion. One-fourth of the 'normal weight' of pulp (the universally adopted 'normal weight' of sugar-containing substance for the polarimetric method of analysis is 26 grams) was digested with one-fourth of the 'constant volume' of lead subacetate solution (the 'constant volume' in the Sachs-Le-Docte process is 177 cc), which was prepared according to the directions given in handbooks of sugar analysis. Pulp samples were obtained with the Keil boring rasp.

Table 2 shows the averages of the results of the sugar analyses of individual beets of 1925, 1926, and 1927. It gives a comparison, with regard to sugar content, of P19 and commercial beets of approximately the same size. The number of analyzed beets was very small in the commercial checks because a high percentage of the plants in the check rows had died or were severely stunted at the end of the season. Rarely a plant had grown to a fair size either because of some accidental combination of favorable factors, or because it possessed a cer-

tain degree of resistance. These occasional individuals were selected and tested for sugar, with the object of using them as new initial selections for resistance.

The unsatisfactory seed germination caused a wide spacing of the beets in most P19 rows. When the area that is available to a beet plant is greater than is necessary for its normal nutrition, vegetative growth is favored and the sugar percentage is reduced. Unsatisfactory stands may have caused a reduction of sugar percentage in the P19 beets. However, since early in the season the check rows also

TABLE 2

AVERAGES OF THE RESULTS OF SUGAR ANALYSES OF INDIVIDUAL BEETS OF THE P19 STRAIN AND OF THE CORRESPONDING COMMERCIAL CHECKS, 1925, 1926, 1927

Location of test plots	Date of seeding	Strain	Number of beets tested	Average weight per beet, grams	Sugar			Range of variation, per cent	
					Average per cent	Grams per beet			
					Lowest	Highest			
Greenfield.....	Mar. 13, 1925.....	P19.....	53	608	15.3	93	9.0	19.2	
		Check.....	10	603	18.5	112	15.6	20.0	
King City.....	Mar. 24, 1925.....	P19.....	191	910	14.7	134	8.0	19.0	
		Check.....	37	859	17.8	153	13.8	20.8	
King City.....	Mar. 29, 1926.....	P19.....	306	773	13.0	100	8.8	17.6	
		Check.....	21	752	13.8	104	10.4	19.4	
King City.....	April 4, 1927.....	P19.....	363	865	12.6	109	9.4	16.5	
		Check.....	20	1064	13.2	140	10.4	19.4	
King City.....	May 5, 1927.....	P19.....	465	984	13.1	129	8.2	19.2	
		Check.....	69	1030	16.1	166	12.6	21.6	

showed deficient stands under the influence of the disease, the values obtained for the sugar percentage of the P19 and the commercial beets can justly be regarded as comparable.

Table 3 presents the results of sugar analyses of composite samples. In addition to the late spring plantings, some seed was sown in 1927 on January 27, i.e., three months before the appearance of the leaf-hoppers. These beets were comparatively old when the inoculation first took place (April 21) and the effect of the disease was not sufficiently strong to reduce the yield of the commercial seed below that of the P19. The test plot of 1928 was also subjected to a mild infection. The April planting of 1927 and the planting of 1929 were very severely affected by curly top. Thus, the data given in table 3 may be grouped in two distinct sets: one comparing the P19 beets and the commercial seed under conditions of mild infection, the other under those of a severe infection.

When the pulp samples were made up the two sets of beet samples had to be treated somewhat differently. In the lots grown under more favorable conditions none of the beets were stunted by the disease, and all could be sampled with the sampling machine. The two other lots, on the other hand, contained some roots which were too small for sampling (below 45 grams in weight). These were eliminated. In calculating the sugar per 100 feet of row, the discarded beets were assumed to have the same sugar percentage as the beets that were tested. When severely affected by curly top, the P19 lots contained on the average 80 per cent of beets suitable for sampling, and the commercial checks 40 per cent.

TABLE 3

SUGAR ANALYSES OF COMPOSITE SAMPLES OF THE P19 AND THE CORRESPONDING CHECK ROWS, 1927, 1928, 1929

Location of test plots	Date of seeding	Strain	Average weight per beet, grams	Sugar		
				Per cent	Grams per beet	Kilograms per 100 feet of row
King City.....	Jan. 27, 1927.....	P19.....	643	15.6	100	9.20
		Check.....	794	17.9	142	13.06
King City.....	April 4, 1927.....	P19.....	425	13.3	56	2.74
		Check.....	225	14.9	34	0.51
Davis.....	March 17, 1928.....	P19.....	725	15.0	109	10.20
		Check.....	718	18.0	129	12.05
Davis.....	March 15, 1929.....	P19.....	492	14.1	69	3.42
		Check.....	264	16.1	43	1.04

The sugar analyses, as given in tables 2 and 3, show that the P19 beets tested consistently lower than the commercial. The sugar percentage varied in both the P19 strain and in the commercial seed. In general, the more severe the test, the lower was the sugar percentage in both strains. Thus, in 1926, 1927, and 1929 the beets tested lower than in 1925 and 1928, and in 1927 the April-planted beets had a lower sugar percentage than the January beets. The difference between the P19 and the commercial seed decreased somewhat with increase of the severity of the test.

It has not been determined whether the increase in the degree of infection alone was responsible for a decrease in sugar percentage. In all probability, in the cases described, this decrease resulted from a combination of several unfavorable factors of growth, one of these being the severe infection with curly top.

Under more favorable conditions the total yield of sugar per 100 feet of row was higher in the commercial beets than in the P19. However, under conditions of severe infection the P19 gave a higher yield in total sugar than the commercial seed.

Conclusions Concerning the P19 Strain.—The P19 strain was found to possess a decided resistance to curly top. Evidently, however, this strain has no commercial value, owing to the following characteristics: (1) the low viability of seed; (2) the reduced vigor; (3) the comparatively low sugar percentage; and (4) the undesirable shape of root.

This strain may, however, be profitably employed in hybridization work. This would involve hybridization of the P19 strain with those of greater vigor, of higher sugar percentage, and with better shape of root. Improvement of the viability of the seed is also expected from such hybridization.

HYBRIDIZATION EXPERIMENTS WITH THE P19 STRAIN

Method of Hybridization.—The hybridization experiments were only of a preliminary nature. Hand-pollination methods were not used. The two beets to be crossed were merely set out together and were allowed to cross-pollinate freely. If one of the two beets sent up seed stalks earlier than the other, these were cut back, and in this manner both beets were forced to bloom simultaneously. The distance between the two plants was only two feet, so that during blooming the branches of the plants were partly intertwined. Furthermore, the seed stalks were frequently shaken during anthesis.

Usually a high percentage of hybrids can be expected in the progenies of beets planted under such conditions. Archimovitch⁽¹⁾ found, in progenies of 17 sugar beets which were distributed among table beets, an average of 90.75 per cent hybrid plants. The lowest percentage of hybrids observed was 70.20. In all probability these values would have been still higher if it were possible to recognize not only the hybrids between the table and the sugar beets, but also those between the sugar beets themselves. Hallquist⁽⁷⁾ analyzed 131 progenies of sugar beets which were exposed to cross-pollination with table beets. They were planted in a field of table beets in such a manner that the distance between the sugar beets themselves was 25 meters, but they were surrounded by table beets. He found cross-fertilization in 98.4 per cent of the cases. The lowest percentage of identified hybrids was 75.6, and the highest, 100.

Individual beets exhibit considerable variation with regard to self-fertility, as has been shown by Grinko.⁽⁶⁾ On the average, however, cross-fertilization is the rule, especially in strains which show high sterility under conditions of self and close-pollination. Sterility was strongly pronounced in the P19 beets which were isolated individually or in groups of closely related plants.

Results of Hybridization.—In the discussion of the results obtained with P19 beets which were exposed to cross-pollination with beets of other origin, no attempt will be made to analyze the inheritance of resistance or any other character. The data at hand are not sufficient for such an analysis. It is possible to show, however, that some definite changes of the characteristics of the P19 strain have been secured.

With regard to improvement of vigor and shape of root, the most successful results were obtained by exposing a P19 beet to cross-pollination with a beet of the California resistant strain developed by Dr. Eubanks Carsner of the United States Department of Agriculture. The two strains show marked morphological differences. The P19 beet roots are short and broad; those of the California, long and tapering. The tops of the P19 are sparse, form a flat rosette, and have a pale green color; those of the California are more dense and erect, and the leaves are of a darker color. Both strains are markedly resistant to curly top, and are low in sugar.

The seed from the two mother plants which were crossed was kept separate. Some of the seed from each mother was planted immediately after harvest, and the stocklings were transplanted in groups and individually in the spring of 1928. Two progenies of the F1 generation (reciprocal crosses) were available for the test of 1928, and several lots of F2 progenies for the test of 1929.

Both plants of the parental generation yielded abundant seed of high viability. The resulting progenies showed a markedly increased vigor. This character was observed during the second as well as during the first year of growth, for the transplanted roots developed unusually vigorous seed stalks, and seed was set abundantly.

With regard to resistance, sugar percentage and morphologic characteristics, the two progenies (i.e., reciprocal crosses) of the F1 generation were similar. The characteristics of the top of the California beet, such as length of petioles, erectness, and darker color, appeared to be dominant. The roots were long, resembling those of the California strain. The sugar percentage was comparable with that of the parent strains.

As the conditions of curly-top inoculation were not favorable in 1928, the reduction in yield in the commercial beets was slight in

comparison with the P19 strain (see tables 1 and 5). The hybrid progenies, however, gave a very much higher yield than either the commercial or the P19 seed, owing to their higher vigor.

Table 4 gives the weight and sugar percentage of the mother plants, and table 5 presents the results obtained with the F1 generation.

The progenies of the F2 generation of the P19 \times California hybrid, which were tested under conditions of severe infection in 1929, showed a remarkably high degree of resistance. They gave a yield not only

TABLE 4
CHARACTERISTICS OF P19 AND CALIFORNIA MOTHER BEETS WHICH WERE USED FOR HYBRIDIZATION

Pedigree No.	Weight of root, grams	Sugar	
		Per cent	Grams per beet
628/26—P19.....	577	16.4	95
814/26—Calif.....	1,615	14.6	236

TABLE 5
RESISTANCE TEST OF THE F1 GENERATION OF THE P19 \times CALIFORNIA HYBRID, 1928

Strain	Roots per 100 feet of row, Kgm.	Average weight per beet, grams	Sugar	
			Per cent	Kilograms per 100 feet of row
P19 ♀ \times Calif. ♂ F1.....	157.69	1,460	14.2	22.39
Calif. ♀ \times P19 ♂ F1.....	140.71	1,393	14.2	19.98
P19 fifth generation.....	67.98	687	15.0	10.20
Checks.....	62.03	634	17.9	11.10

very much higher than the commercial checks, but also higher than any other resistant strain. Furthermore, while every beet in the commercial checks and in all other strains, including the P19, showed symptoms of curly top, the hybrid progenies had a high percentage of beets which failed to develop the symptoms. A possible explanation of this condition is that the curly-top virus might have been extremely attenuated in the symptom-free beets. Cases of such extreme attenuation of the virus in resistant beets have been reported by Lackey.⁽⁸⁾

Table 6 presents the results obtained with the F2 generation. Plate 1 shows the behavior of the F2 progenies under the severe infection of 1929. Figures 3, 4, 5, and 6 show the effect of hybridization upon the shape of the P19 root.



Fig. 3. Types of roots of the P19 strain. The three types represented on the right-hand side occur more commonly than the one on the extreme left.

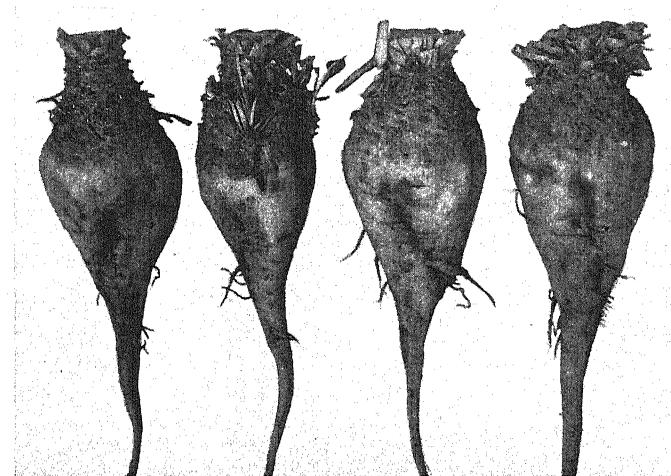


Fig. 4. Types of roots of the California strain.

Tables 5 and 6, figures 3 to 6, and plate 1 show that the progenies obtained through hybridization of the P19 with the California strain are far superior to the P19 strain with regard to vigor and to shape of root.

With the object in view of increasing the percentage of sugar in the P19 strain, a P19 beet was exposed to cross-pollination with a beet of the strain 651-3 produced by Dr. Dean A. Pack of the United States Department of Agriculture. This strain was developed for high yield and high sugar percentage and without reference to curly top. Incidentally it was found to be somewhat resistant to curly top.

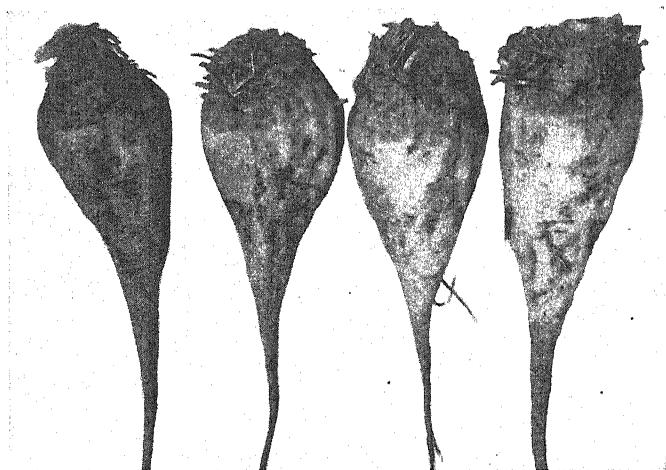


Fig. 5. Types of roots of a P19 ♀ × California ♂ progeny.

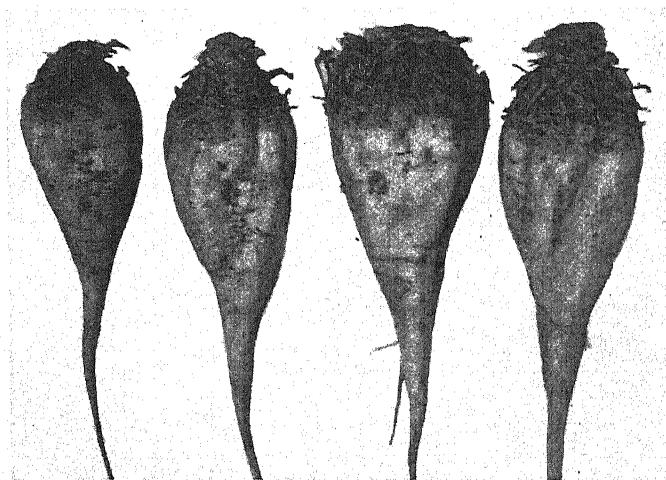


Fig. 6. Types of roots of a California ♀ × P19 ♂ progeny.

Table 7 shows the characteristics of the mother plants, and table 8 presents in a preliminary way the results of the resistance test of the F1 generation.

Table 8 shows that the P19 × 651-3 hybrid progenies of the two reciprocal crosses have a marked degree of resistance and a high sugar percentage. A comparison of this table with tables 2 and 3 shows that these hybrid progenies are far superior to the P19 with regard to sugar percentage.

TABLE 6

RESISTANCE TEST OF THE F₂ GENERATION OF THE P19 × CALIFORNIA HYBRID, 1929

Strain	Number of beets per 100 feet of row		Percentage of beets that survived	Percent- age of beets that remained free from symptoms	Beets per 100 feet of row, Kgm.		Average weight per beet, grams		Sugar	
	On May 8	Athar- vest			Tops	Roots	Tops	Roots	Per cent	Kilo- grams per 100 feet of row
	P19 ♀ × Calif. ♂ F ₂	140	134	96	67	31.98	136.40	239	1018	13.7
Calif. ♀ × P19 ♂ F ₂	140	129	92	53	32.34	112.95	251	876	12.7	14.34
P19 ♀ × Calif. ♂ F ₁	128	124	97	54	43.95	151.55	355	1222	12.8	19.14
Average of checks.....	139	55	40	0	0.87	7.32	16	133	16.2	1.19
P19 fifth generation.....	76	69	91	0	7.59	24.25	110	351	14.1	3.42
Average of checks.....	138	49	36	0	1.67	6.47	34	132	16.1	1.04

TABLE 7

CHARACTERISTICS OF P19 AND 651-3 MOTHER BEETS WHICH WERE USED FOR HYBRIDIZATION

Pedigree No.	Weight of roots grams	Sugar	
		Per cent	Grams per beet
1973/27—P19.....	1,928	11.0	212
1975/27-651-3.....	1,162	17.0	198

TABLE 8

RESISTANCE TEST OF THE F₁ GENERATION OF THE P19 × 651-3 HYBRID

Strain	Number of beets per 100 feet of row		Percent- age of beets that survived	Beets per 100 feet of row, kgm.		Average weight per beet, grams		Sugar		
	On May 8	Athar- vest		Tops	Roots	Tops	Roots	Per cent	Kilograms per 100 feet of row	
	1973/27-P19 ♀ F ₁	133	130	98	8.75	33.16	67	255	17.2	5.70
1975/27-651-3 ♂ F ₁										
1975/27-651-3 ♀ F ₁	144	130	90	8.07	43.09	62	332	17.3	7.46	
1973/27-P19 ♂ F ₁										
Average of checks.....	145	65	45	1.99	8.53	31	131	15.8	1.35	

MASS SELECTIONS FOR RESISTANCE

Seed Production.—Additional selections for resistance in commercial fields and in commercial check rows in the trial plots were carried out each season. The mother beets of the later selections were treated *en masse*, because suitable locations for the isolation of seed beets were scarce, and most of them were reserved for the P19 strain. The initially selected beets were planted together and were allowed to cross-pollinate freely within the group. However, the seed from individual mother beets was kept separate. In the succeeding generations the mother beets were grouped according to size and sugar percentage, but were not hooded.

It was found that most of the beets of initial selections did not produce any seed; and those that did go to seed yielded, with a few exceptions, progenies which showed at least some degree of resistance when compared with an ordinary commercial strain. Carsner and Stahl⁽⁶⁾ call attention to the fact that curly top materially affects the production of beet seed. Curly-top-diseased beets either fail to go to seed in the second year or produce only dwarfed, diseased stalks. The probability is that in the case of the initial selections from severely diseased beets in commercial fields, the majority of beets which did not possess an inherent resistance failed to go to seed. Accidental factors may have influenced the growth of such individuals during the first year, making them appear resistant regardless of the presence of symptoms upon the foliage. In the second year, after transplanting, however, such beets in most cases would show the effect of the disease by developing severe symptoms and by failing to go to seed normally. A practice was adopted to aid this second natural selection for resistance by eliminating before anthesis those individuals which produced only dwarfed or small seed stalks.

Table 9 shows the behavior of the different selections for resistance during the second year of growth. Apparently the development of seed stalks in the second year in curly-top-infected beets is correlated with resistance.

It will be noted that the percentage of seed stalks was higher in the initial selections from check rows than in the initial selections from commercial fields. The reason is that a more severe selection for resistance was made among the beets of the check rows because they were planted under conditions most favorable for the development of curly top.

Test for Resistance of Mass-selected Progenies.—The progenies of the later selections were tested for resistance together with the P19 strain and under similar conditions. With the exception of 1928, the

TABLE 9

PERCENTAGE OF SEED STALKS DEVELOPED NORMALLY DURING THE SECOND YEAR OF GROWTH IN VARIOUS STRAINS SELECTED FOR RESISTANCE

Kind of selection	Year of selection	Number of beets selected	Beets which produced normal seed stalks	
			Number	Per cent
Initial selections from commercial fields.....	1919	5,000	183	3.7
	1923	3,000	24	0.8
	1924	1,176	101	8.6
	1925	472	25	5.3
Initial selections from check rows.....	1925	38	13	34.2
	1926	12	3	25.0
	1925 (P23)	50	31	62.0
Second selections.....	1926 (P24)	143	82	57.3
	1925 (P19)	219	185	84.5
Third selections.....	1926 (P19)	76	64	84.2

TABLE 10

RESULTS OF THE RESISTANCE TEST OF MASS-SELECTED PROGENIES (1925 TO 1929)

Location of test plots	Date of seeding	Strain	Generation	Number of progenies	Number of beets per 100 feet of row		Percentage of beets that survived	Roots per 100 feet of row, kgm.	Average weight per beet, grams
					Early in season	At harvest			
Greenfield.....	Mar. 13, 1925	P23.....	First.....	8	93			13.17	142
		Check.....			74			5.75	78
King City.....	Mar. 24, 1926	P24.....	First.....	101	41			9.79	239
		Check.....			10			0.76	76
King City.....	April 4, 1927	P24.....	First.....	16	63	50	79	16.07	321
		Check.....			66	33	50	2.65	80
King City.....	April 4, 1927	P25.....	First.....	24	81	61	75	16.64	273
		Check.....			71	26	37	1.83	70
Davis.....	Mar. 17, 1928	P24.....	Second....	36	111	108	97	115.76	1,072
		Check.....			113	111	98	128.30	1,156
Davis.....	Mar. 21, 1929	P25.....	Second....	18	130	110	85	33.26	302
		P19 check.....			95	86	91	21.51	250
		Commercial check.....			141	48	34	7.05	147

conditions for curly-top infection were favorable every year. In 1928 leafhoppers were not disseminated upon the mass-selected progenies, and as they were removed some distance from the plots where nymphs were distributed, they became infected very late and to no appreciable



Fig. 7. Resistance test of mass-selected progenies. Row 1, resistant mass-selected progeny. Row 2, commercial check. Row 3, resistant mass-selected progeny. King City, September 26, 1927.



Fig. 8. The same progenies as in figure 7 shown at harvest time. The respective yields per 100 feet of row were: (1) 21.55 kgms; (2) 0.91 kgms; (3) 22.23 kgms. King City, October 18, 1927.

extent. Table 10 presents the results obtained with the mass-selected progenies, and figures 7 and 8 show the effect of curly top upon two of the progenies and the corresponding check row.

Sugar Analyses of the Mass-selected Progenies.—The beets of the initial selections were not analyzed for sugar. Their size and shape were the two principal characteristics upon which the selection was

based. In the first filial generation, however, the future mother beets were also tested for sugar. The analyses were conducted according to the same methods as were followed in the case of P19 beets. Tables 11 and 12 below give the results of the analyses.

TABLE 11

AVERAGES OF THE RESULTS OF SUGAR ANALYSES OF INDIVIDUAL BEETS OF THE FIRST FILIAL GENERATION OF MASS-SELECTED BEETS AND OF THE CORRESPONDING COMMERCIAL CHECKS

Year	Strain	Number of beets tested	Average weight per beet, grams	Sugar	
				Per cent	Grams per beet
1925.....	P23.....	61	628	18.1	114
	Check.....	10	603	18.5	112
1926.....	P24.....	409	706	14.4	102
	Check.....	10	690	15.6	108
1927.....	P24.....	85	1,003	14.1	141
	P25.....	131	934	15.4	144
	Check.....	19	1,053	14.3	151

TABLE 12

SUGAR ANALYSES OF COMPOSITE SAMPLES OF THE MASS-SELECTED PROGENIES AND OF THE CORRESPONDING COMMERCIAL CHECKS

Location of test plots	Date of seeding	Strain	Generation	Average weight per beet, grams	Sugar	
					Per cent	Kilograms per 100 feet of row
King City.....	April 4, 1924	P24.....	First.....	421	14.6	2.81
		P25.....	First.....	376	15.0	1.90
		Check.....		190	14.8	0.31
Davis.....	Mar. 17, 1928	P24.....	Second.....	1,056	16.6	19.22
		Check.....		1,163	16.5	21.17
Davis.....	Mar. 21, 1929	P25.....	Second.....	463	16.5	5.49
		P19 check.....		334	13.8	2.97
		Commercial check.....		318	16.0	1.13

Results Obtained with the Mass-selected Progenies.—Some of the progenies of mass-selected beets showed a considerable degree of resistance in the first and in the second generations. Owing to better germination and better stands, they gave in most cases a higher yield than the average of the P19 progenies. The percentage of surviving beets was higher and the average size of roots larger than in the commercial checks.

Unlike the P19 strain, the mass-selected progenies showed little uniformity with regard to the degree of resistance and to morphological characteristics. The plants, even within the individual progenies, showed variation in the severity of symptoms developed, and in many cases the most advanced symptoms could be observed in some beets of the most resistant progenies. When exposed to infection the mass-selected progenies showed a lower percentage of surviving beets and usually a smaller average size of roots than the P19.

The sugar percentage was found to be as high in these strains as in the commercial checks. The total sugar content was considerably higher than in the commercial beets, when the trial was conducted under exposure to infection.

SUMMARY

1. The breeding of sugar beets for resistance to the curly-top disease was conducted for five years.
2. Plants that were apparently less injured by the disease in severely infected commercial sugar beet fields served as initial selections for the development of resistant strains.
3. The seed obtained from such beets was tested for resistance, together with ordinary commercial seed under exposure to a severe infection with curly top.
4. Resistance to curly top was found to be an inherent characteristic of many individual beets selected in commercial fields.
5. In the case of one of the most resistant strains, designated as P19, a consistent transmission of this characteristic through five successive generations was observed.
6. When grown under exposure to infection, the P19 strain gave a higher yield, a higher percentage of surviving beets, and a higher average size of roots than the commercial strain (R. & G. Old Type), with which it was compared.
7. Immunity was not observed in the P19, or in any other resistant strain; the P19 beets, however, developed as a rule only the transparent venation, which was discernible only with some difficulty.
8. The resistance of the P19 strain was found to be relative; under exposure to more severe conditions of testing for resistance the percentage of surviving beets decreased somewhat and the yield became lower, although it was always much higher than in the corresponding check rows.

9. With regard to sugar analyses, the P19 strain tested usually 2 to 3 per cent lower than the commercial beets which were grown under similar conditions. When not exposed to a severe infection with curly top, the total sugar per unit area was higher in the commercial beets; when subjected to a severe infection the P19 strain gave a much higher yield of total sugar per unit area than the commercial.

10. The P19 strain was found to be of no value for commercial purposes owing to (1) the low viability of seed, (2) the low sugar content, (3) the reduced vigor, and (4) the undesirable shape of the root.

11. With the object in view of improving the undesirable characteristics of the P19 strain, some hybridization experiments have been started. Cross-pollination between P19 and another resistant strain, of greater vigor and with a better shape of root, gave very resistant progenies, which also showed marked increase in vigor and a better shape of root. This hybrid, however, was low in sugar. Cross-pollination between the P19 and a strain less resistant but high in sugar, gave resistant progenies which showed an increase in sugar percentage in comparison with the P19.

12. In addition to the work with the P19 strain, selections of resistant mother beets in the commercial plantings were carried out repeatedly for several years and were propagated *en masse*. The progenies of these mother beets were compared with ordinary commercial seed and with the P19 under exposure to infection with curly top.

13. The mass-selected progenies were markedly resistant to curly top, although less uniform with regard to this characteristic than the P19 strain.

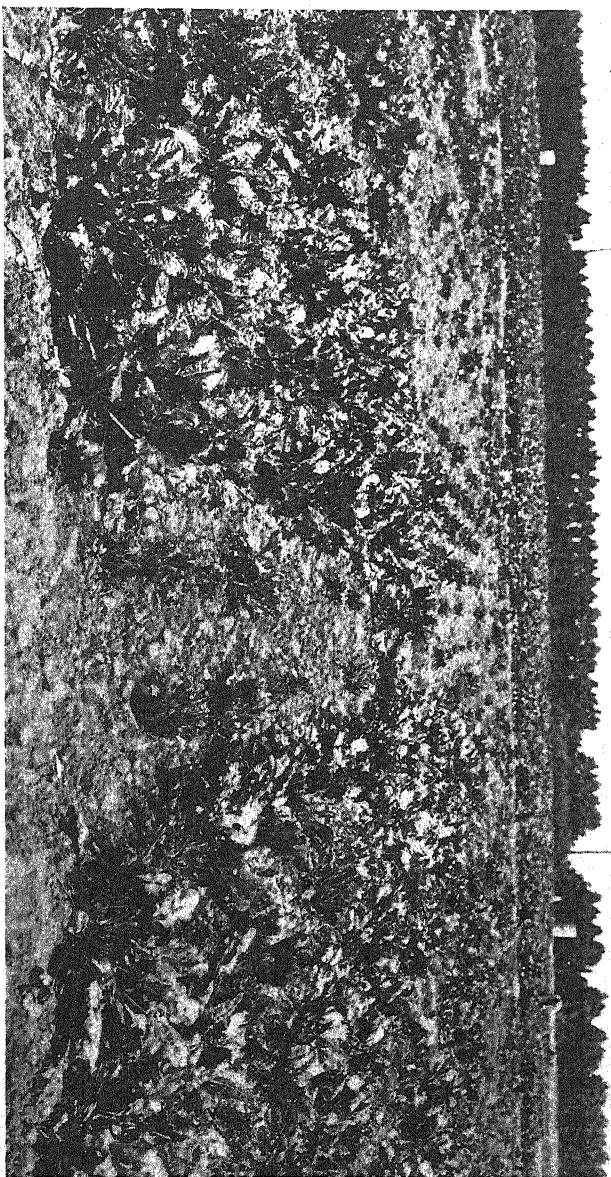
14. The mass-selected progenies were as high in sugar percentage as the commercial checks with which they were compared.

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The P19 ♀ × California ♂ hybrid progenies (4 rows left and right) and commercial beets (2 rows in the center) under exposure to a severe infection with curly top. Davis, California, July 8, 1929.

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ELECTROPHORESIS OF TOBACCO MOSAIC VIRUS¹

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INTRODUCTION

Iwanowski's⁽³⁴⁾ demonstration in 1892 that the mosaic disease of tobacco is caused by a filter-passing agent opened a new and extensive field of research in the pathology of both plants and animals. Since then a number of different virus⁴ diseases have been found affecting a variety of plants and animals. The bacteriophage has also been considered a virus by many workers.

Much literature dealing with various phases of virus diseases has been published. In the following review we have attempted to abstract only the work giving evidence as to the nature of the viruses.

REVIEW OF LITERATURE

Cytological Studies.—For some time pathologists resorted to cytological methods in their search for a visible parasite causing the virus diseases. A great deal of careful work has been done in this field. Iwanowski,⁽³⁵⁾ Dickson,⁽²¹⁾ McKinney, Eckerson and Webb,⁽⁴⁵⁾ Goldstein,⁽³⁰⁾ Palm,⁽⁵⁴⁾ Rawlins and Johnson,⁽⁵⁸⁾ Hoggan,⁽³²⁾ Kunkel,⁽³⁸⁾ Holmes,⁽³³⁾ Smith,⁽⁶¹⁾ and various students of animal pathology have described abnormal intracellular bodies in virus-infected host tissues. The structure of these bodies is not sufficiently distinct to warrant the conclusion that they are a stage of a causal organism. In fact, many of the workers are inclined to the view that they are products of the causal agent or of the diseased host cell.

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⁴ In this paper a virus is considered to be a filter-passing agent capable of causing an infectious disease.

Size of Virus Particles.—Iwanowski⁽³⁴⁾ and Beijerinck⁽⁸⁾ discovered independently that the infective agent of tobacco mosaic passed through filters which intercepted ordinary bacteria. Beijerinck assumed that the virus was in the form of a "*contagium vivum fluidum*." Since then the size limits of tobacco mosaic virus particles have been studied by Duggar and Karrer.⁽²³⁾ Using a series of ultra-filters graded by means of organic particles of known dimension the size of the virus particles was found to be comparable to that of haemoglobin, having a diameter of approximately $30\mu\mu$.

Olitsky, Traum and Schoening⁽⁵²⁾ employing similar methods estimated the size of the virus particles of foot and mouth disease to be somewhere between 20 and $100\mu\mu$.

Prausnitz,⁽⁶⁵⁾ using similar technique estimated the size of Flexner bacteriophage as approximately $20\mu\mu$.

From the above results it appears that we cannot hope to observe the virus particles by ordinary microscopic methods. The presence of colloidal particles of approximately this size has been detected by use of the ultramicroscope in the hands of students of colloidal chemistry. However, until we are able to separate a virus from the colloids found in plant juices or animal fluids we cannot hope to detect or distinguish the virus particles by this method. Numerous attempts have been made to detect motile organisms in virus-infected fluids by means of the dark-field microscope but in general such attempts have been unsuccessful.

Dilution.—It is known that the virus of tobacco mosaic withstands great dilution without losing its infective properties. Allard⁽²⁾ showed that infective tobacco juice diluted one part to a thousand is quite as effective in producing the disease as the undiluted juice, but that when diluted one part to ten thousand the percentage of infection is reduced. In some cases positive results were found even in dilutions as high as one part to one million. These results indicate that the concentration of virus in plant juice is very high.

Longevity.—Tobacco mosaic virus can be dried for a relatively long period without losing its disease-inciting properties. Chapman⁽¹⁶⁾ finds 3 years, Beijerinck⁽⁸⁾ and Walker⁽⁶⁷⁾ 2 years, and Allard⁽¹¹⁾ 18 months as its period of longevity.

There is considerable difference in the longevity of other viruses causing plant diseases. Walker⁽⁶⁷⁾ reports that tomato mosaic virus when dried at air temperature remained active 30 days, ground cherry virus 23 days, and cucumber mosaic virus less than 24 hours. However, when these viruses were kept in their plant extracts some of them remained infectious for a much longer period of time, tomato mosaic

virus remaining infectious 138 days, ground cherry virus 69 days, and cucumber mosaic virus from 24 to 48 hours.

Hastings⁽³¹⁾ reports that spores of anthrax bacillus remained viable for 18.5 years in a naturally infected sample of pond water. This period is about forty-two times as great as that noted by previous observers working with spores in artificial culture. "A member of the colon aerogenes group" isolated from a corn plant and then placed on filter paper kept at 37° C remained viable 31 days. The same organism when left on corn stover withstood desiccation for a period of 7 years. Thus it is evident that certain bacteria are even more resistant to drying than are the viruses.

Cultivation of Virus.—Although the viruses causing mosaic diseases multiply very rapidly in living plant tissues they have never been satisfactorily cultivated in artificial media.

Olitsky⁽⁵⁰⁾ used fresh juice from healthy tomato plants as a medium and produced infection up to the twelfth subplant. "By the fourth subplant the original subplant was present in an estimated dilution of one to ten million." This exceeds the dilution limit of all known viruses. From these results he concluded that he had cultivated the virus in an artificial medium.

Following the publication of Olitsky's findings four other investigators, Mulvania,⁽⁴⁶⁾ Goldsworthy,⁽²⁹⁾ Purdy,⁽⁵⁶⁾ and Smith⁽⁶²⁾ repeated his experiments in an attempt to confirm his results. All their results were negative, however.

Purdy⁽⁵⁷⁾ demonstrated that tobacco mosaic virus multiplies in freshly detached tobacco leaves placed petiole downward in moist sand. Under such conditions symptoms do not appear.

Thermal Inactivation.—As compared with the vegetative stage of most microorganisms certain viruses have a relatively high thermal inactivation point. In this property these viruses are similar to enzymes. Bertrand and Thomas⁽¹⁰⁾ state that, "'Nearly all enzymes are completely inactivated by short exposure to a temperature of 100° C.'" The inactivation of saccharase and blood catalase has been found by Willstatter, Graser and Kuhn⁽⁶⁸⁾ to be in a great measure dependent on the degree of purity of the given enzyme.

Mulvania⁽⁴⁷⁾ reports that a temperature of 80° C for three days reduced the activity of tobacco mosaic virus 40 per cent.

Allard's work⁽⁸⁾ indicates that a temperature of 90° C or above for five minutes inactivated the virus.

The thermal death-point of the vegetative stage of most microorganisms lies between 37° and 55° C. Some of the viruses also have thermal inactivation points in this range. Esty⁽²⁶⁾ found that resistant

spores of *Bacillus botulinus* under optimum conditions of growth withstood a temperature of 100° C for 330 minutes, 110° C for 33 minutes and 120° C for 4 minutes.

It was found by McKinney⁽⁴⁴⁾ that the thermal inactivation point was depressed approximately 6° C when infective juice of mosaic tobacco plants was diluted one part to one hundred.

Air-dried mosaic tobacco leaves were found by Allard⁽³⁾ to retain their infectivity after being heated at 100° C for one-half hour, while infective extract which had been evaporated to dryness was easily inactivated by this treatment. These results indicate that the system in which the virus is contained has a distinct influence upon its thermal inactivation point.

Allard⁽³⁾ has demonstrated that low temperatures do not inactivate the virus of tobacco mosaic, —12° C for one to four hours or repeated freezing and thawing failing to inactivate it. Even the temperature of liquid air, —180° C, did not inactivate it.

Johnson's work⁽³⁶⁾ indicated that optimum temperature for the development of mosaic symptoms in tobacco is similar to that which is optimum for bacterial growth, namely around 30° C. This temperature is lower than that most favorable for the activity of most enzymes, the optimum temperature for enzymes, according to Waksman and Davison,⁽⁶⁶⁾ usually falling between 40° and 50° C. At 35° C symptoms of tobacco mosaic failed to develop.

Johnson's results are very interesting but unfortunately are open to several interpretations. It is conceivable that the metabolism of the host plant may have been altered sufficiently by the high temperature to produce conditions in the host cells which are unfavorable for the rapid multiplication of the virus and that inhibition of symptoms may not have been due to the direct influence of high temperature on the virus.

In general, it appears that temperature studies have yielded little evidence as to the nature of the viruses.

Action of Chemicals.—Viruses in general are relatively resistant to chemicals which are toxic to most organisms. Allard^(1, 3) found that common disinfectants such as phenol, cresol, thymol, camphor and naphthalene have little effect on tobacco mosaic virus. Even formaldehyde below concentrations of 4 per cent did not readily inactivate the virus. Salts of heavy metals such as lead nitrate and mercuric chloride had little effect on certain viruses, a 1 per cent solution of mercuric chloride failing to inactivate tobacco mosaic virus in 33 days. Strong acids such as nitric and hydrochloric acids reduced infectivity only in concentration approaching 1 gram in 50 to 100 cc

of infective juice. Phosphoric, citric and acetic acids were without effect except in concentrations of 1 gram in 20 to 50 cc of infective juice. Hydroxides of aluminum and nickel tended to form precipitates and leave the supernatent liquid without infectious properties. In the case of aluminum hydroxide the virus was found to be in the precipitate and was recovered from it. It is interesting to note here that aluminum hydroxide is a positive colloid and would be expected to precipitate negative colloids. Nickel ions seemed to have a definite toxic effect upon the infective principle, destroying it entirely. In this solution nickel hydroxide was present in a concentration of 2 grams in 1000 cc of infective juice. When juice from mosaic tobacco plants was passed through tale or kaolin the filtrate was found to be free of virus.

The American Commission for the Investigation of the Foot and Mouth Disease,⁽⁵²⁾ working with the virus of the foot-and-mouth disease of animals, found that sodium hydroxide had no coagulating action on the lymph containing the virus and reported it as being virucidal in concentrations of 1 to 2 per cent acting for 1 minute.

Allard⁽⁵³⁾ found that 1 gram of sodium hydroxide in 1000 cc of virus solution inactivated tobacco mosaic virus after 2 days.

He further found that green mosaic tobacco leaves are quickly killed by ether and chloroform vapor but the infective principle contained in them is uninjured. Toluol and carbon tetrachloride are also without effect on the virus.

Allard showed that ethyl alcohol added to filtered plant extract containing tobacco mosaic virus throws down a precipitate carrying the virus with it. When alcohol of 45 to 50 per cent strength was used the supernatant liquid was free of virus. However, higher concentrations, 75 to 80 per cent, inactivated the virus in the precipitate.

The virucidal action of alcohol on the same virus taken from mosaic tomato was investigated by Smith.⁽⁶²⁾ The virus was not inactivated by alcohol at as high a concentration as 90 per cent. The supernatant liquid remained active after treatment with 70 per cent alcohol and the residue still retained its full activity after subjection to 90 per cent alcohol. Even when precipitation was prevented by the addition of sodium hydroxide, 90 per cent alcohol failed to inactivate the virus.

Vinson and Petre⁽⁶⁵⁾ find that precipitation of the virus from the juice of mosaic tobacco plants is fairly complete when two volumes of acetone or alcohol are added to one volume of infective extract. Such a precipitate was found to be highly infective when suspended in water.

Olitsky, Traum and Schoening⁽⁵²⁾ indicated that the action of alcohol on the foot-and-mouth virus is largely prevented by the coagulum formed around the virus particles. They showed that filtered lymph containing active virus is less resistant to alcohol than the unfiltered lymph and regard this difference as due to the smaller amount of protein present in the former. When coagulation by alcohol was prevented by the addition of a small amount of sodium hydroxide the alcohol was found to have a greater virucidal action.

On the other hand, Bronfenbrenner⁽¹³⁾ working with bacteriophage gave evidence that the apparent inactivation of a bacteriophage by alcohol is a surface phenomenon rather than that of disinfection by the penetration of the alcohol into an organism. He has demonstrated that in the case of an unpurified bacteriophage the major portion of the lytic principle is found in the precipitate formed on the addition of alcohol. Bronfenbrenner then purified the bacteriophage as far as possible by growing the bacteria on a synthetic medium with ammonium salts as the only source of nitrogen. A lytic filtrate of very high potency was obtained. This filtrate was then fractionated and freed of all dialyzable material by means of electro ultra-filtration. After hydrolysis a residue was obtained which appeared to be free from ammonia when tested with Nessler's reagent. This indicates the absence of proteins in the bacteriophage. When the purified bacteriophage was treated with 10 volumes of alcohol precipitation did not occur and no inactivation of the lytic principle took place after remaining in this solution at 22° to 25° C for eight days. Thus it appears that in this case the alcohol has little direct influence on the virus.

Duggar and Armstrong⁽²⁴⁾ found that expressed juice from healthy pokeweed plants is capable of inactivating the virus of tobacco mosaic. They suggest that the action is probably due to the adsorption of the virus by the colloids of the foreign plant juice.

Elmer⁽²⁵⁾ found that extracted juice from healthy bean, cucumber, and tobacco plants reduced the infective power of tomato mosaic. This effect, however, was only temporary, full infectivity being regained in 21 days.

The results of the above workers show that viruses may resist certain powerful disinfectants but are inactivated by numerous relatively inert colloids. Such results indicate that colloids in the medium may be of great importance in inactivating the viruses or in their influence on inactivation by disinfectants. It is apparent, therefore, that a virus must be purified before its reaction to various chemicals can be determined with certainty.

The relatively great resistance of certain viruses to toxic chemicals is apparently evidence favoring their non-living nature.

Ultra-Violet Radiation.—Bovie⁽¹¹⁾ showed that ultra-violet radiation of wave length shorter than $292.5\mu\mu$ killed bacteria and spores of fungi in 10 minutes.

It was found by Smith⁽⁶¹⁾ that although *Bacillus prodigiosus* suspended in distilled water is inactivated by ultra-violet light in 30 seconds, tobacco mosaic virus in filtered plant extract is permanently inactivated only after continuous exposure for 30 minutes to such light. As shown by the following workers the differential absorption of the effective rays by the two media, distilled water and plant extract, may have been responsible for at least part of the difference observed by Smith.

Arthur and Newell⁽⁵⁾ used samples of purified virus prepared by the acetone precipitation method outlined by Vinson and Petre.⁽⁶⁵⁾ Drops of the purified tobacco mosaic virus were distributed on a glass plate and covered with a quartz plate about $\frac{1}{8}$ inch thick. The irradiation took place through the quartz plate at a distance of 15 inches. Such a preparation of tobacco mosaic virus was found to be completely inactivated by 15 seconds exposure to the open arc. Virus applied to surface of tobacco leaves could be inactivated by ultra-violet radiation but after the virus had penetrated the plant, inactivation could not be made to take place.

McKinley, Fisher and Holden⁽⁴³⁾ studied the influence of ultra-violet light on bacteria, a bacteriophage, and viruses. They state that under the conditions of their experiments, "a lytic principle active for *Bacterium coli* 'D' is acted upon by ultra-violet light in much the same way as are two strains of known filterable viruses, i.e., herpes and Levaditi's so-called encephalitis virus. Exposure to ultra-violet light at a distance of one foot for forty minutes is sufficient to attenuate or destroy both the bacteriophage and the two filterable viruses employed in these experiments." *B. coli*, however, was apparently unaffected under the same conditions. In a substrate of normal rabbit serum the bacteriophage and the two filterable viruses are protected from the action of ultra-violet light.

Baker and Nanavutty⁽⁶⁾ considered the opacity of beef broth to ultra-violet rays in studying the effect of such rays upon bacteriophage. They found that beef broth in the proportion of 1:1000 in normal saline produced inappreciable opacity to the bactericidal light rays when the depth of the exposed solution was less than 1 cm. Because a quantitative comparison of the results obtained by various workers is difficult due to differences in methods and lack of a unit

measurement of the bactericidal output of a mercury vapor lamp these investigators took the value 1 as time required to destroy *B. coli* and made relative measurements of the inactivating effect of ultra-violet rays upon several bacteriophages and enzymes. Their results are as follows: *B. coli* 1, Shiga bacteriophage about 2, staphylo bacteriophage 1 to 2, trypsin and complement 20 to 30, haemolytic amboceptor 50, diastase and lysozyme 120. From this they conclude that the susceptibility of bacteriophage to ultra-violet rays is similar to that of bacteria.

Because of the different results obtained by the above workers no general conclusions can be drawn as to the relative resistance of organisms, viruses, and enzymes to ultra-violet light.

The Influence of X-Rays on Viruses.—Mulvania⁽⁴⁷⁾ found that X-rays do not inactivate tobacco mosaic virus but that attenuation results when the exposure is lengthened.

Attenuation.—It has been observed by many virus investigators that attenuation of viruses may take place under certain conditions. Mayer⁽⁴²⁾ found that continued heating at 60° C did not change perceptibly the infective power of tobacco mosaic virus but subjection to a temperature of 65° to 75° C caused attenuation.

Other studies on the attenuation of tobacco mosaic virus were made by Johnson.⁽³⁷⁾ Virus-inoculated tobacco plants were placed in a constant temperature chamber at a temperature between 35° and 37° C for ten or more days. At this temperature mosaic symptoms are wholly or partially masked. Virus from these plants produced a mild form of the disease in inoculated plants. Repeated serial transfers from such plants to healthy tobacco plants did not perceptibly alter the attenuated condition of the virus.

Carsner,⁽¹⁵⁾ working with the virus causing curly top of sugar beet, reports that the passage of this virus through *Chenopodium murale*, *Rumex crispus* and *Suaeda moquinii* causes the virus to become attenuated so that a mild form of curly top develops when the virus is transferred to healthy beets.

Organisms are known to be attenuated by certain unfavorable conditions. Consequently this behavior may be regarded as evidence of the living nature of a virus.

Respiration.—Carbon dioxide is produced during respiration in most organisms. Bronfenbrenner⁽¹³⁾ by the aid of a sensitive micro-respirometer attempted to detect the generation of CO₂ by a bacteriophage and by the herpes and rabies viruses. After being confined for 48 hours in the apparatus under both anaerobic and aerobic conditions neither bacteriophage nor viruses gave a positive test for the presence

of carbon dioxide. From these results it appears that the virus either has a very unusual type of metabolism in which carbon dioxide is not given off, as is reported by Palladin⁽⁵³⁾ for acetic acid and glycerine bacteria and for sorbose bacteria by Bertrand,⁽⁶⁾ that respiration is so slight that the carbon dioxide is not detectable; or a third alternative, that the virus is non-living and therefore does not respire.

Influence of Oxygen.—Johnson⁽³⁶⁾ finds that the longevity of tobacco mosaic virus is shorter in well-aerated sand and sandy soils than in heavier clay and organic soils. When exposed in a moist condition to an excess of oxygen the virus is sensitive to its inactivating influence. Cleveland⁽¹⁸⁾ has shown similar behavior in protozoa. On the other hand, Bayliss⁽⁷⁾ states that the enzyme rennet can be inactivated by passing air through the solution.

Purification of Virus.—It seems clear that in some cases the true properties of the viruses have not been determined, the results having been greatly influenced by the constituents of the medium. A knowledge of this relation of the virus properties to environment makes apparent the importance of purifying the viruses as much as possible before attempting to determine their properties.

Arnold and Weiss⁽⁴⁾ separated a bacteriophage, lytic to *Bacillus typhosus*, from antigenic bacterial proteins derived from dissolved bacteria. A precipitation of antigenic bacterial proteins was effected by adding a 14 per cent solution of Na_2SO_4 . Digestion with trypsin was also found to give satisfactory results. A 1 per cent trypsinized bacteriophage solution was incubated for 48 hours, filtered through paper and then passed through a Berkefeld candle. The absence of antigenic bacterial proteins was indicated by the failure of the supernatant liquid and filtrate to produce agglutinins against the corresponding bacteria.

Bronfenbrenner⁽¹⁸⁾ and De Groat⁽²⁰⁾ also purified bacteriophages until no test for proteins was obtained.

Among investigators of viruses causing plant diseases Vinson and Petre⁽⁶⁵⁾ have attempted to purify tobacco mosaic virus by using a number of different methods.

They succeeded in removing some of the phosphates, sulfates, and most of the proteins and pigments from the extract by using low concentrations of lead acetate and barium acetate. Safranin was found to be very effective in precipitating the virus, 100 cc of a 1 per cent safranin solution added to 50 cc of infective juice causing a precipitate to form and leaving a supernatant liquid almost free of virus. The virus was then released from the safranin by adding amyl alcohol. Attempts were also made to salt out the virus by means of ammonium

sulfate. The precipitate, however, contained salts, proteins, and pigments as well as the virus.

Heat fractionation was tried. Juice from mosaic Turkish tobacco was separated into two heat-precipitable fractions, one around 85° C and the other above 90° C. The first fraction contained an appreciable amount of nitrogen and was still infective after the heating, although the virus concentration was greatly reduced. Boiling the juice rendered it non-infectious.

Brewer, Kraybill, and Gardner⁽¹²⁾ attempted to purify tomato mosaic virus by centrifuging and clearing with aluminum gel. They succeeded in obtaining a very clear and active preparation of the virus.

Sherman, Caldwell, and Adams⁽⁵⁹⁾ purified pancreatic amylase until it was very active in dilutions as great as 1:100,000,000. They consider that even the most delicate protein test cannot detect proteins in concentrations less than 1:10,000 and that the failure of certain workers to obtain a protein test from their purified enzyme preparations may be due to the very low concentration of enzyme in the solutions tested. It is possible that the above criticism may be applicable to work on the viruses. Certainly failure to detect proteins in a purified virus or bacteriophage should not be considered as proof of the absence of proteins unless a very concentrated preparation of virus or bacteriophage has been used.

Electrophoresis.—In their experiments on electrophoresis, Olitsky, Traum, and Schoening⁽⁵²⁾ found an isoelectric range of the foot-and-mouth virus around pH 8; below pH 8 the virus carried a positive charge and above this pH a negative charge. Thus the virus appeared to behave like a protein having an isoelectric point around pH 8.

Todd⁽⁶⁴⁾ demonstrated that a bacteriophage lytic to *Bacillus dysenteriae* Shiga isolated from chicken droppings migrated to the anode between pH 3.6 and 7.6. He failed to find an isoelectric point as the phage showed signs of inactivation at lower pH values.

Douglas and Smith⁽²²⁾ found that vaccinia virus migrates to the anode in the range pH 5.5 to 8.4. They further found that the isoelectric point of the tissue protein was pH 6.8. Therefore, on the alkaline side of its isoelectric point the protein migrated with the virus to the anode but on its acid side the protein migrated to the cathode.

By electrophoresis Olitsky and Long⁽⁵¹⁾ obtained active virus at the anode from the serum of vaccinia-recovered rabbits. This serum had given no infection when used as an inoculum before electrophoresis. They believe that electrophoresis concentrated the virus at the anode.

Vinson and Petre⁽⁶⁵⁾ reported that the virus of tomato mosaic migrated to the negative electrode at pH 4.76.

Until the electrophoresis of more viruses is studied we cannot draw conclusions as to the similarity of their electrophoretic behavior or as to the significance of such behavior.

The migration of bacteria in an electric field has been studied by many investigators. Winslow, Falk, and Caulfield⁽⁶⁹⁾ found *Bacillus cereus* to be isoellectric at pH 3, positively charged between pH 3 and 1, and isoelectric at pH 1.

Winslow and Shaughnessy⁽⁷⁰⁾ found one isoelectric point of *Bacillus cereus* and *Bacterium coli* near pH 3.0 and another around pH 13.5.

Pneumococcus (variant type 1) was shown by Falk and Jacobson⁽²⁷⁾ to be charged negatively above pH 3.3 and positively between pH 2.7 and pH 0. At pH 0 another isopotential point was approached.

Falk, Sharp, and Link⁽²⁸⁾ found that bacteria from the smooth colonies of *Bacterium phaseoli sojense* were positively charged between pH 1.4 and pH 2.8 and negatively charged between pH 3.1 and 10. Bacteria from rough colonies showed a positive charge between pH 1.2 and 2.6 and a negative charge above pH 2.8.

Northrup and de Kruif⁽⁴⁸⁾ found that the bacillus of rabbit septicemia (type D) had a negative charge above pH 3, was isoelectric at pH 3, and had a positive charge at pH 2.2. Another isoelectric point was observed around pH 1.1. Another strain (type G) was isoelectric around pH 4.2 and was positively charged at pH 2, which was the lowest pH value tested. *Bacillus typhosus* was found to be isoelectric around pH 3, positively charged from pH 3 to around pH 1, and isoelectric below pH 1.

Addition of egg albumin or globin to the bacillus of rabbit septicemia was found by Northrup and de Kruif⁽⁴⁹⁾ to change the isoelectric point of this organism to approximately that of the added protein.

In general it appears that bacteria usually have a negative charge at pH values between 3 and such high values as 13. An isoelectric point is commonly found around pH 3 and the organisms usually have a positive charge in the region between pH 3 and 1. Around pH 1 another isoelectric point has usually been found.

When having a positive charge the organisms usually migrate toward the cathode at a comparatively slow rate, indicating a low potential at the surface of the organisms. The most rapid movement toward the anode is usually at higher pH values. Rapid migration toward this pole is usually observed between pH 6 and 9.

All of the studies on electrophoresis of viruses have been made at relatively high pH values. Further studies should be made at low pH values in order to compare the electrophoretic behavior of viruses with bacteria at pH values where the bacteria show characteristic changes.

Since Northrup and de Kruif⁽⁴⁹⁾ have shown that the addition of sufficient protein confers the electrophoretic behavior of the protein on bacteria, and Loeb⁽⁴⁰⁾ has shown a similar action of proteins on negative colloids such as collodion particles, one may well suspect that a virus which shows electrophoretic behavior similar to a protein is exhibiting the properties of adsorbed protein rather than those of the virus.

Summary of Available Evidence on the Nature of the Viruses.—Most of the studies made on virus properties have not yielded strong evidence as to whether the viruses studied were living or inanimate. It is of course possible that all of the viruses are not similar in nature. In fact the so-called "incubation periods" observed in the transmitting insect and host plant by Smith and Bonequet⁽⁶³⁾ and Severin⁽⁶⁰⁾ in the case of curly top of sugar beet, and by Kunkel⁽³⁹⁾ in the insect transmitting aster yellows suggests that these viruses may be heterogenous organisms and may be quite different from most of the mosaic viruses.

Perhaps the strongest evidence favoring the theory that the mosaic viruses are organisms is furnished by their susceptibility to attenuation, while the strongest evidence favoring their inanimate nature is apparently their great resistance to certain chemicals. The apparent absence of protein in the bacteriophage may also be considered as evidence against the phage being a living organism.

The most promising type of work for gaining evidence on the nature of the viruses would appear to be an attempt at purification of the viruses and a study of the properties of the purified viruses. Bronfenbrenner,⁽¹⁴⁾ Vinson and Petre,⁽⁶⁵⁾ and Brewer, Kraybill, and Gardner⁽¹²⁾ have already begun this type of work and have made considerable progress.

Electrophoresis should be very helpful in purifying a virus and in giving evidence as to its nature.

If electrophoretic behavior of a virus at different pH values were known it should be possible to separate the virus from much of the colloidal material in the plant juice by causing the migration of the virus to one pole while much of the other colloidal material fails to move or migrates to the opposite pole. For example the bacteriophage

studied by Todd which had a negative charge at pH 3.6 would migrate to the anode at this pH while most, if not all, of the proteins in solution would migrate to the cathode, since most proteins have an isoelectric point above this pH.

With the exception of the single observation made by Vinson and Petre,⁽⁶⁵⁾ no studies on the electrophoresis of viruses causing plant diseases have been reported. It appeared to us that a study of the electrophoretic behavior of plant viruses might yield evidence as to the nature of such viruses, and as to their similarity to bacteria and to the viruses causing animal diseases. Furthermore, such a study of the electrophoretic behavior of a virus at various pH values is necessary if one is to proceed intelligently in an attempt to use electrophoresis or precipitation in purifying a virus. In the following pages we are reporting electrophoretic studies made on the virus causing tobacco mosaic.

EXPERIMENTAL WORK

Methods.—For the purpose of our experiment the Todd⁽⁶⁴⁾ U tube electrophoresis apparatus was selected. To prevent polarization of the electrodes, agar bridges filled with 1 per cent KCl in 2 per cent agar connected each limb of the U tube to electrode vessels containing a saturated solution of CuSO₄. Strips of copper plate were used for electrodes.

Buffer solutions of pH 9 to pH 3 were made according to Clark.⁽¹⁷⁾ Those having pH below 3 were made of potassium phthalate, of phosphoric acid adjusted with hydrochloric acid, or contained only hydrochloric acid.

The plant extract containing the virus was derived from tobacco plants showing characteristic mosaic symptoms. The original virus was kindly supplied by Dr. James Johnson of the University of Wisconsin. A meat grinder was used to crush the leaves and the juice was expressed by pressing the pulp in a piece of cotton cloth. The extract was then centrifuged, passed through a Berkefeld "V" candle under aseptic conditions, and the filtrate collected in sterile test tubes. The pH of this filtrate was found to be 5.6.

Extreme care was taken to adjust the filtrate to the same pH as the buffer in the two arms of the apparatus. This was done by adding approximately one part of the buffer solution of the desired pH to two parts of the plant extract and then dropping small quantities of 1/5 normal HCl or NaOH into the solution from a fine pipette until the pH was adjusted.

In the first set of experiments, the results of which are shown in table 1, a direct current circuit giving 108 volts was used. The current varied from 4 to 9.5 milliamperes and the time from 3 to 5 hours. In later experiments, the results of which are shown in table 2, two or three radio "B" batteries giving 96 to 145 volts supplied the current, which varied from 6 to 9 milliamperes. The duration of electrophoresis in these experiments was 5 to 7.5 hours.

After electrophoresis, samples were taken from each limb of the U tube. These samples are designated in the tables as "anode" and "cathode" respectively. "Bulb" refers to the sample of adjusted plant extract taken from the central bulb and serves as a check on the activity of the virus. A portion of each of these samples was then tested to determine the change in pH during the course of the experiment. All determinations were made by means of the hydrogen electrode except at pH 1.2. At this pH the hydrogen electrode was found to give erratic results and the colorimetric method was therefore substituted. The remainder of each sample was used for inoculating tobacco plants.

Inoculations in the first set of experiments were made by the ordinary needle puncture method. In the second set young leaves of the tobacco plant were rubbed with a small piece of cotton cloth saturated with inoculum. The latter method was found to give much more consistent results.

Results.—The results of the experiments are shown in tables 1 and 2. These results indicate that the virus carries a negative charge when in solutions having a pH between 4 and 9 but that the virus is either uncharged or has a very low P.D. when in solutions having a pH between 3 and 1.2.

Only one plant inoculated with liquid from the cathode developed mosaic symptoms. Since the virus migrated to the anode so consistently at pH 4 and failed to migrate to the cathode at all lower pH values it appears that the infection of this plant must have been accidental. In fact, it is rather surprising that more inconsistencies did not arise since the mosaic disease of tobacco is so easily transmitted during watering and handling of the plants.

TABLE 1

INFLUENCE OF THE pH ON THE DIRECTION OF MIGRATION OF TOBACCO MOSAIC VIRUS;
FIRST EXPERIMENT

pH before electro- phoresis	Inoculum	Milli- amperes	Volts	Time	pH after electro- phoresis	Number of plants inoculated	Number of plants infected
				hours			
1.0	Cathode.....	6-8	108	4	1.15	4	0
1.0	Anode.....	6-8	108	4	1.3	4	0
1.0	Bulb.....	6-8	108	4	2	1
2.0	Cathode.....	5.0	108	4	2.0	4	0
2.0	Anode.....	5.0	108	4	2.2	4	0
2.0	Bulb.....	5.0	108	4	2	0
3.0	Cathode.....	4.0	108	4	3.0	4	0
3.0	Anode.....	4.0	108	4	3.0	4	0
3.0	Bulb.....	4.0	108	4	2	0
4.0	Cathode.....	6.5	108	5	4.05	4	0
4.0	Anode.....	6.5	108	5	4.1	4	0
4.0	Bulb.....	6.5	108	5	2	0
4.0	Cathode.....	4.0	108	4	4	1
4.0	Anode.....	4.0	108	4	4	0
4.0	Bulb.....	4.0	108	4	2	1
5.0	Cathode.....	7.5	108	5	5.0	4	0
5.0	Anode.....	7.5	108	5	5.0	4	2
5.0	Bulb.....	7.5	108	5	2	1
6.0	Cathode.....	6.5	108	3	6.2	4	0
6.0	Anode.....	6.5	108	3	6.2	4	3
6.0	Bulb.....	6.5	108	3	6.2	2	2
7.0	Cathode.....	7.5	108	5	7.0	4	0
7.0	Anode.....	7.5	108	5	7.2	4	2
7.0	Bulb.....	7.5	108	5	2	1
8.0	Cathode.....	6.75	108	3	8.2	4	0
8.0	Anode.....	6.75	108	3	8.2	4	1
8.0	Bulb.....	6.75	108	3	7.8	2	1
9.0	Cathode.....	9.5	108	5	9.0	4	0
9.0	Anode.....	9.5	108	5	9.0	4	2
9.0	Bulb.....	9.5	108	5	9.0	2	1

TABLE 2

INFLUENCE OF THE pH ON THE DIRECTION OF MIGRATION OF TOBACCO MOSAIC VIRUS;
SECOND EXPERIMENT

<i>pH</i> before electro- phoresis	Inoculum	Milli- amperes	Volts	Time	<i>pH</i> after electro- phoresis	Number of plants inoculated	Number of plants infected
				<i>hours</i>			
1.2	Cathode.....	9.0	96	7.5	1.3	10	0
1.2	Anode.....	9.0	96	7.5	2.0	10	0
1.2	Bulb.....	9.0	96	7.5	1.4	10	10
1.8	Cathode.....	8.0	145	5.0	2.1	5	0
1.8	Anode.....	8.0	145	5.0	2.6	5	0
1.8	Bulb.....	8.0	145	5.0	2.1	2	2
2.1	Cathode.....	8.5	145	7.5	2.5	5	0
2.1	Anode.....	8.5	145	7.5	2.8	5	0
2.1	Bulb.....	8.5	145	7.5	2.55	2	2
3.0	Cathode.....	6.0	96	7.5	3.0	5	0
3.0	Anode.....	6.0	96	7.5	3.05	5	0
3.0	Bulb.....	6.0	96	7.5	3.2	2	2
4.0	Cathode.....	7.0	145	7.5	4.05	5	0
4.0	Anode.....	7.0	145	7.5	4.1	5	5
4.0	Bulb.....	7.0	145	7.5	4.1	2	2
4.5	Cathode.....	7.5	145	7.5	4.49	5	0
4.5	Anode.....	7.5	145	7.5	4.5	5	5
4.5	Bulb.....	7.5	145	7.5	4.5	2	2
5.0	Cathode.....	7.5	145	7.5	4.9	5	0
5.0	Anode.....	7.5	145	7.5	5.1	5	5
5.0	Bulb.....	7.5	145	7.5	5.0	2	2
6.0	Cathode.....	8.0	140	5.5	5.95	5	0
6.0	Anode.....	8.0	140	5.5	5.90	5	5
6.0	Bulb.....	8.0	140	5.5	6.0	2	2
8.0	Cathode.....	8.0	145	5.0	6.8	5	0
8.0	Anode.....	8.0	145	5.0	7.0	5	5
8.0	Bulb.....	8.0	145	5.0	7.0	2	2
9.0	Cathode.....	8.0	145	5.0	8.90	5	0
9.0	Anode.....	8.0	145	5.0	8.95	5	5
9.0	Bulb.....	8.0	145	5.0	9.0	2	2

DISCUSSION

From the results obtained it appears that electrophoresis at pH 4 should separate the virus from at least some of the proteins present in the plant extract. Cohn, Gross, and Johnson⁽¹⁹⁾ gave evidence that certain of the proteins in tomato juice have an isoelectric point around pH 5 and it is probable that some of the proteins in tobacco also have isoelectric points above pH 4. Such proteins would migrate to the cathode at pH 4 and would therefore be separated from the virus, which migrates to the anode at this pH.

The fact that the virus did not migrate to the cathode at low pH values indicates that the virus did not adsorb sufficient protein to modify its electrophoretic behavior and suggests that the observed electrophoretic behavior of the virus is similar to that which would be exhibited by the pure virus.

An electrophoretic behavior such as that observed would be expected if the surface of the virus particles were composed largely of compounds which are weak acids. Such compounds would exist as dissociated salts at high pH values, the anion on the surface of the particle giving it a negative charge. At lower pH values such compounds would be changed to undissociated weak acids which would not confer a charge on the surface of the particles.

According to Bayliss⁽⁷⁾ "practically all inert substances are negative to pure water. In acid solution they are either positive or less negative than in pure water, while in alkaline solutions the negative charge is increased."

Loeb⁽⁴¹⁾ studied the electrophoretic behavior of a number of kinds of chemically inert particles such as mastic, graphite, gold and collodion. In concentrations of HCl below M/128 these particles had a negative charge. In M/128 to M/32 HCl a slight positive charge was produced on mastic, graphite, and gold, but collodion remained negative even in M/8 HCl.

The electrophoretic behavior of bacteria as discussed earlier in the paper is very similar to that of the particles of mastic, graphite, and gold studied by Loeb.

Since viruses and bacteriophages have been considered by numerous workers to be non-living and enzymic in nature it may be of interest to briefly discuss the evidence upon this point which is afforded by the electrophoretic behavior of enzymes. According to

Waksman and Davison⁽⁶⁶⁾ invertase is isoelectric at pH 5, catalase at pH 7, trypsin at pH 10.2. These isoelectric points are all higher than is usually found for bacteria.

Tobacco mosaic virus exhibited an electrophoretic behavior very similar to that of bacteria and chemically inert particles, the only difference being in the failure of the virus to migrate to the cathode at pH values below 3.

The failure of the virus to migrate to the cathode at low pH values is of questionable significance. Most bacteria that have been studied have carried a relatively weak positive charge at pH values around 1 to 3. Winslow, Falk, and Caulfield⁽⁶⁹⁾ found that the most rapid movement of *B. cereus* toward the cathode was 2.8 microns per second at pH 1.5 while the most rapid migration toward the anode was at pH 7.2 when a migration velocity of 10.6 microns per second was attained. It is possible that the virus may have carried a weak positive charge at certain of the pH values at which it appeared to be isoelectric but that its rate of migration was so slow that it was not detected.

Several workers have shown that the migration of bacteria toward the cathode may be inhibited by certain salts in the solution. For example, Winslow, Falk, and Caulfield⁽⁶⁹⁾ found that the addition of .363M NaCl prevented the migration of *B. cereus* toward the cathode. Thus it appears that the potential at the surface of bacteria when they tend to migrate toward the cathode is so slight as to be easily inhibited by various constituents in the surrounding medium. It therefore appears that we cannot consider the failure of tobacco mosaic to migrate to the cathode as evidence that it is of a different nature from bacteria.

Our results do not agree with those of Vinson and Petre,⁽⁶⁵⁾ who reported that tobacco mosaic virus migrated to the negative electrode when contained in a concentrated tomato extract having a pH of 4.76. The reason for this difference in results is not evident.

SUMMARY

Unpurified tobacco mosaic virus migrated to the anode during electrophoresis between pH 4 and pH 9. No migration of the virus was detected between pH 3 and 1.2.

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MASS PRODUCTION OF EGG PARASITES OF THE GENUS TRICHOGRAMMA¹

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INTRODUCTION

Of the comparatively small number of entomophagous insects whose habits and host relations appear to make them usable in biological control work, the hymenopterous egg-parasite, *Trichogramma*, has probably received the widest attention. Attempts have been made to utilize it in every phase of biological control, by protection through proper cultural practices, by introduction to establish it in new habitats, and by production in quantities for mass liberations. The liberation of *Trichogramma* in great numbers may prove to be a practical method for the control of several species of injurious insects.

The following account gives the origin and development of the mass production³ of *Trichogramma* and the methods of utilizing it.

Trichogramma minutum Riley was used in this work because it is the most common representative of the genus in California. The characteristics that adapt it for mass production are probably generic.

CHARACTER OF TRICHOGRAMMA

The genus *Trichogramma* was established by Westwood⁽²⁵⁾ in 1833. The type specimens were found on an oak leaf in Epping Forest. There are few distinct species. The species most frequently

¹ The material for this paper was collected, for the most part, while author was employed by the Satieoy Walnut Growers' Association, Satieoy, California.

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³ The first, and so far only, successful application of the mass-production idea occurred in California. There a method was developed for utilizing *Cryptolaemus montrouzieri* Muls., an introduced coleopterous predator of the citrophilus mealybug.⁽²³⁾

encountered in Europe and America are *T. evanescens* Westw. and *T. minutum* Riley. The latter is not well differentiated morphologically from *T. evanescens*. In America the genus was first recorded when Lintner⁽¹³⁾ reared *Trichogramma minutum* from saw-fly eggs at Utica, New York, in 1866.

The adult parasite is less than 1 mm in length and is brightly colored. Its wings are refringent and appear in direct sunlight a brilliant purple, its eyes are bright red, and its body varies from dark brown to clear yellow.

This chalcid is widely distributed, and its hosts, according to Martin,⁽¹⁵⁾ number well over one hundred and fifty species in the orders Lepidoptera, Coleoptera, Hymenoptera, Neuroptera, Diptera, and Hemiptera. It has been known to attempt oviposition in globules of okra juice, the swollen abdomen of the mite, *Pediculoides* sp., paper smeared with the hair covering of the egg masses of the brown-tail moth, and in the dry excrement of adult moths. The majority of its hosts, however, are lepidopterous, and its oviposition is usually confined to eggs in exposed places unprotected by a hard or sticky covering. Eggs on a sticky or very pubescent surface are relatively immune to attack. The highest number of individuals developing in a single hymenopterous host egg was noted by Severin and Severin⁽²¹⁾ who found thirty pupae in an egg of *Cimbex americana* Leach. Patterson⁽¹⁷⁾ reared thirty-seven adults from a single egg of *Coloradia pandora* Blake, a lepidopterous host.

According to Howard and Fiske⁽¹²⁾ there are two "races" of the *minutum*-like form of *Trichogramma* which differ only in that one is thelytokous and the other arrhenotokous. The former is reported only from Europe. Marchal⁽¹⁴⁾ found, in addition, color differences and no interbreeding, and suggested that if observations were made from various hosts and localities a number of strains of *Trichogramma* would be found having the status of races or elementary species. Girault⁽⁷⁾ states that undoubtedly "biological species" exist. The races of *Trichogramma* when reared under identical environmental conditions may possibly be differentiated by the length of their life cycles and amount of pigmentation in the adult females.

The life cycle from egg to adult varies according to the host and with the prevailing temperature. The shortest life cycle of *Trichogramma* observed by the writer occurred in the eggs of *Ephestia cautella* Wlk. At temperatures between 91°-95° F it completed its life cycle in 144 hours. The pupal stage was reached 72 hours after oviposition.

After about one-third of the time required for the completion of the life cycle has elapsed, the host eggs begin to turn dark and finally become more or less black. This change is brought about in the eggs of all host species by preparation of the larva for pupation. In flattened types of eggs, such as the eggs of *Alabama* and *Carpocapsa*, after the enclosed parasites have formed their pupae, the shell sometimes shrinks about them, leaving little oval cells indicating the position of the parasites. When ready to emerge the parasite cuts a hole in the shell just large enough for it to struggle through. *Trichogramma* does not void meconium until after emergence. At emergence it is sexually mature.

Light intensity appears to be the dominating factor in the activity of *Trichogramma*. Emergence from the host egg is stimulated by light; in confinement it is positively phototropic; an increase in light intensity appears to stimulate copulation, and a decrease in light intensity retards activity. Locomotion ceases when light intensity is low.

Howard and Fiske⁽¹²⁾ found that *Trichogramma* can be reared from infertile eggs or eggs killed by cold storage, but not from eggs in which the embryological development has passed beyond a certain point prior to attack or from eggs very often stung.

ADAPTABILITY OF *TRICHOGRAMMA* TO BIOLOGICAL CONTROL WORK

Thirty-five years ago Enock⁽⁴⁾ called attention to the possibilities in 'farming' *Trichogramma* on a large scale. Recent investigations show that it is adapted to this work in many ways. Some of the biotic characteristics that determine its adaptability are as follows:

1. It mates and oviposits readily in confinement.
2. It develops to maturity in the eggs of moths which feed in stored grain.
3. It has a short life cycle.
4. Its development extends throughout a greater temperature range than many of its hosts.
5. It has a great variety of hosts.
6. It accommodates itself as to number of generations according to the host it parasitized, as ascertained by Marchal.⁽¹⁴⁾
7. It develops throughout the year, temperature and food permitting.
8. It has few competing species and no known secondary parasites.

9. It maintains a concentration sufficient to effect a local reduction of the host. Dispersal is so localized that its effectiveness is measurable.

Factors that limit to some extent its adaptability are as follows:

1. It does not appear to be specific as to host location; it apparently finds its host by means of random movements, tending to climb upward and fly downward.
2. It will deposit more eggs in a host than can develop to maturity.
3. It will oviposit in eggs too far developed to enable the parasite to mature.
4. Under natural conditions while in the host egg it is prey to such predators as ants, ladybird beetles, and mites.

ABUNDANCE OF *TRICHOGRAMMA* AS AFFECTED BY FARM PRACTICES

Cultural practices such as the burning of sugar-cane trash⁽¹¹⁾ and the flooding of cranberry bogs⁽⁶⁾ appear to reduce the abundance of *Trichogramma* the following spring. Experiments in the maintenance of *Trichogramma* in the sugar-cane fields of Louisiana through the conservation of cane trash have been conducted during the past ten years, but proof of its hibernation in cane trash is lacking. *Trichogramma* dies within its host when the latter remains submerged in water. During the submergence of its host, however, it may develop to the late pupal stage.

Clean cultivation, by reducing the number and variety of the food plants of the hosts of *Trichogramma*, acts as a check on the natural abundance of the latter. In southern California there is a noticeable difference between the degree of parasitism of the codling moth in orchards and on fruit trees in dooryard situations where food plants of moths are present throughout the year. Properly managed cover crops may aid parasitism under orchard conditions.

As a rule, the hosts of *Trichogramma* are widely distributed general feeders and adaptive successful insects that have a large excess of progeny. As a group, they are benefited by the attack of this parasite, since it tends to prevent them from completely using up their food supply. Under natural conditions a very high degree of parasitism often occurs late in the season when the host is abundant. Since the excess of progeny is greatest in plant environments as modified by man, *Trichogramma* may be of value in maintaining such pest populations below the economic zero.

EARLY METHODS OF INCREASING PARASITE POPULATION

Owing to the extreme variability of its food supply the appearance of *Trichogramma* under natural conditions is seasonal and irregular. Organized production as a means of overcoming this handicap captured the imagination of entomologists in Europe and America. The objective of this early work was to advance the date of effective abundance by accelerating the natural accretion with liberations made early in the season.

In 1909 Howard and Fiske⁽¹²⁾ liberated a great many thousands against the brown-tail moth. The parasites were held in cold storage in brown-tail moth eggs. These eggs had been collected in the field the previous summer and then parasitized.

The habit of the brown-tail moth of depositing its eggs in masses prevents control by *Trichogramma*, for the parasite can attack only the outer eggs. Howard and Fiske concluded, however, that since it is an efficient parasite on numerous other hosts it would, at some future time, be possible to utilize it. Mokrzecki and Bragina⁽¹⁶⁾ in 1913 found that the number of *Trichogramma* which it is possible to rear in the laboratory is theoretically unlimited. Mokrzecki, however, cautioned the Russian fruit growers against exaggerated expectation in the results of the artificial production of this parasite and its use against the codling moth.

The earliest method of laboratory host production was that used by Pospelow⁽¹⁸⁾ who reared larvae from the winter imagos of *Euxoa segetum* (Schiff.) on sprouted wheat and slices of potato. Later Portchinsky⁽¹⁸⁾ suggested collecting large quantities of overwintering larvae or pupae of a prolific host of *Trichogramma*, forcing their early maturity and oviposition, and parasitizing their eggs with *Trichogramma* carried over for that purpose from the preceding season. He advocated the use of *Phalera bucephala* L., since the overwintering pupae could be purchased cheaply.

Later it was found that the eggs of *Ephestia kuehniella* Zell. were suitable for the reproduction of *Trichogramma* and were more readily obtained. In Japan *Ephestia* has been used recently to produce *Trichogramma* for use against the rice moth. The susceptibility of *Ephestia* to larval parasitism and the webbing habit of the larva tended to limit its use in quantity production.

Another method proposed by Vuillet⁽²⁴⁾ and also by Harland⁽⁸⁾ was that of increasing the abundance of hosts on the windward side

of infested plantings. For example, *Mamestra brassicae* L. was suggested as a preliminary host adjacent to a vineyard infested with *Polychrosis botrana* Schiff. Den Doop⁽³⁾ states that the spread of the parasite is most probably due to the midday winds. If such is the case, such a method has possibilities.

A natural condition somewhat similar occurred in southern California in 1926 when the codling moth was found to be heavily parasitized as early as the middle of May. The butterfly, *Vanessa cardui* L., had occurred in great numbers during March and April and had deposited quantities of eggs on weeds everywhere. It appears probable that this butterfly served as a preliminary host to *Trichogramma*. Whatever the preliminary host may have been, two hundred out of three hundred codling moth eggs on one walnut tree were found to be parasitized prior to May 25.

INITIATION OF MASS PRODUCTION

In April, 1925, Harry S. Smith had suggested to the writer that *Trichogramma minutum* was the most adaptable egg parasite with which to attempt the biological control of the codling moth since it "is well adapted to this purpose and breeds with extreme rapidity." Consequently, when its natural effectiveness was manifested so strikingly in 1926, the possibility of using it in control work was given serious consideration.

The failure of other workers to accomplish results indicated that success could be attained only by the low-cost production of great quantities of *Trichogramma* so that it could be made effective in the field through sheer force of numbers.

With this idea in mind experimentation began August 11, 1926. Ten females reared from tortricid eggs secured in the field on walnuts were allowed to oviposit in the eggs of *Ephestia kuehniella*. The second to fourth generations were reared on the lichen moth, *Illicia nexa* Boisd., and the potato tuber moth, *Phthorimaea operculella* Zell. The fifth generation was reared on *Sitotroga cerealella* Oliv., which was selected for further experimentation because it was available in large numbers and had the convenient habit of ovipositing in narrow crevices, forming an egg mass of one layer. The use of this moth as laboratory host made possible the solution of the problem of the mass production of *Trichogramma*.

The eggs of *Ephestia cautella* were tested by the writer in Mexico but proved to be unsuitable for mass production, although *Tricho-*

gramma in several instances showed a marked preference for them. The larvae from eggs escaping parasitism spun a dense webbing over the adjacent eggs and also fed on them.

During October about a million silk-worm eggs were donated by the American Silk Factors, Inc., at San Diego for testing as a laboratory host of *Trichogramma*. Although the silk-worm egg proved to be attractive, the tough chorion prevented oviposition. Ten females were observed on one egg, each drilling vigorously in an effort to oviposit.

EFFECT OF HC T ON THE PARASITES

Directly or indirectly the host influences the size, longevity, and fecundity of the adult parasite. In an egg maturing only one parasite, the size of the mature parasite is determined by the size of the egg. When the egg is super-parasitized its size only determines the number of parasites developing in it. A single adult reared from a potato-tuber-moth egg has a larger body than an adult from a *Sitotroga* egg, but in length they are equal. In the field where one to four parasites develop in a codling-moth egg the single parasite may be twice the length of the smallest of the four parasites. The larger the parasite the more young it is capable of producing. *Trichogramma* reared on *Sitotroga* eggs were allowed to oviposit in the large eggs of an arctiid moth. Two of their offspring produced 240 progeny on *Sitotroga*, whereas the maximum progeny per female in successive generations on *Sitotroga* was 45.

It is doubtful, however, if large eggs will prove to have any advantage in artificial production over an equal number of small eggs because of super-parasitism. A *Sitotroga* egg rarely yields more than one *Trichogramma*. Larger eggs which allow the development of more than one parasite may produce under laboratory conditions parasites smaller and weaker than those from *Sitotroga* eggs. If the shell of the host egg is relatively hard they may be unable to emerge.

The longevity of parasites reared on the eggs of *Ephestia cautella* appears to be much shorter than those reared on *Sitotroga*.

A dependable source of an enormous amount of host material is necessary for maximum production. On October 1, 1928, the writer secured three female parasites from codling moth eggs in the Lloyd-Butler orchard at Saticoy. At the end of seven weeks the progeny of these, composing the sixth generation, amounted to about 300,000. The supply of moth eggs available was inadequate to maintain such multiplication.

ADAPTABILITY OF *SITOTROGA* TO THE MASS PRODUCTION OF *TRICHOGRAMMA*

Sitotroga cerealella proved to be peculiarly adapted to the quantity production of *Trichogramma*. Numerous generations can be reared in rapid succession from stored grain under regulated conditions.

The life cycle of *Sitotroga* in the laboratory is about 28 days and its fecundity at least 50 eggs per female. A high rate of reproduction can therefore be obtained.

The habits and tropisms of *Sitotroga* and their value in the mechanical manipulation of production are as follows:

The newly hatched larvae are negatively phototropic and positively geotropic so that corn in bulk can be easily infested. The feeding and the pupal stages occur within the kernels of grain so that the interspaces are not clogged with frass and webbing. The newly emerged moths therefore have free egress from the deeper layers of grain. Incidentally, this grain has a higher sales value after being used than grain matted with webbing and excreta.

The newly emerged moths are as flexible in their movements as are larvae and at first are negatively geotropic and thigmotropic so that they all issue from compactly arranged tilted bins. They return to the corn to oviposit but do not remain there. No dead moths are found when the bins are emptied.

The moths mate promptly after emergence and their pre-oviposition period, as Simmons and Ellington⁽²²⁾ observed, is less than 24 hours under laboratory conditions. Maximum oviposition occurs within 60 hours after emergence, so that the moths can be handled in crowded egg-deposition cages wherein the adult life is shortened to two or three days.

During the daytime moths tend to crawl upward and come to rest in positions of positive thigmotropism and negative phototropism. Loose boards resting against vertical surfaces serve as 'traps.'

The oviposition responses of the females facilitate the collection of eggs. Crevices about 0.23 mm or 0.01 inch in width stimulate egg deposition. By crowding the bottom of the egg cages with moths their bodies form crevices that stimulate egg deposition and the constant shifting causes the eggs to drop through the 20-mesh screen into a trough below.

The chorion of a *Sitotroga* egg is relatively tough so it can be handled in mass as readily as grains of rice. The egg is of a size that prevents, as a rule, the development of more than one *Trichogramma*. In mass production the average size of the parasites probably is greater than would be the case if either larger or smaller host eggs were used.

Under normal conditions *Sitotroga* oviposits only in crevices but the pressure from a strong current of air directed against a 20-mesh screen can be substituted. The positive anemotropic females come to rest where the air velocity is optimum, thrust their ovipositors through the mesh against the air current, and extrude their eggs, which either adhere to the screen or fall free. Egg deposition is thus obtained by a forced draft. The eggs may be prevented from hatching and held for long periods before being parasitized. According to Back⁽¹⁾ their exposure to 1° F for 24 hours prevents hatching.

Sitotroga has relatively few enemies; bacterial and fungoid diseases are rare. In common with other insects, however, it suffers from the ravages of the mite *Pediculoides ventricosus* Newport. A large mite, *Tyroglyphus* sp., feeds to some extent on the eggs, the flat grain beetle, *Cryptolestes pusillus* Schon., will attack the newly hatched larva, and *Dibrachys boucheanus* Ratzeburg parasitizes the pupae. *P. ventricosus*, however, was the only one of these to interfere with production in this experiment.

THE CHRONOLOGICAL DEVELOPMENT OF REARING METHODS⁴

The development of rearing methods progressed rapidly because of the favorable habits and tropisms of the moths.

In August, 1926, about 500 pounds of infested corn was secured from a neighboring grower and placed in shallow bins. In this preliminary work the moths were collected in small glass vials, about a half-dozen to the vial. Since the female normally oviposits only in crevices, two slips of cardboard held together by metal clips were placed in each vial. The eggs were deposited in masses of a single layer which adhered to the cardboard. Each day the cardboards were removed and placed in vials containing newly emerged parasites.

The outside surfaces of the bins and the entire inside surface of the rearing room was painted green in order that the moths at rest on

⁴ The writer is indebted to P. F. Wright, Laboratory Assistant during May, June, and July, 1927, for many helpful suggestions.

them could be easily observed. Early in the experiment the amount of infested corn was increased to nearly 2 tons and was placed in nine bins built one above the other 5½ inches apart (fig. 1). Each bin measured 12 feet long, 3 feet wide, and 3 inches deep. During October, 1926, in order to handle the increase in moth production, 1-gallon battery jars were adopted for egg deposition and halves of petri dishes inverted on square pieces of stiff cardboard for parasite cages.

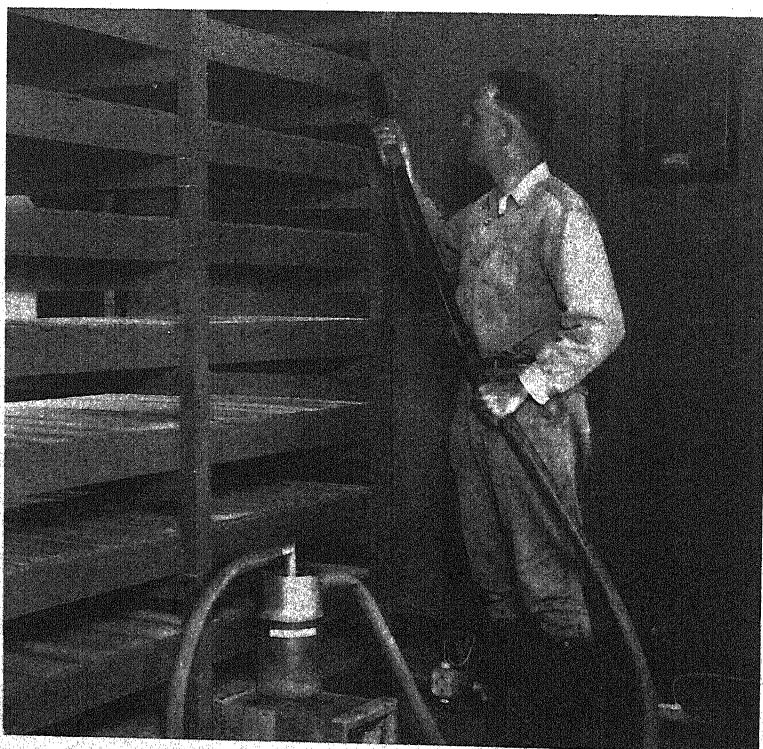


Fig. 1. Type of corn bins used in 1927. Note the heating unit at the rear. (Photo from Los Angeles Times.)

By the first of May, 1927, the daily production of *Trichogramma* amounted to about 25,000. The equipment and method of handling them in use was as follows:

1. 30 egg-deposition cages consisting of
 - (a) 30 1-gallon cylindrical glass battery jars (numbered consecutively).
 - (b) 30 celluloid cones fitted to corresponding jars and also numbered. (The numbers were written with ink and covered

- thinly with shellac.) The cones were made from sheet celluloid and held in shape with celluloid cement. Each cone was about $6\frac{1}{2}$ inches in height and open at each end, the diameter at the small end being 1 inch and that of the large end equaling the inside diameter of the jar.
- (c) 30 circular celluloid grates $3\frac{1}{2}$ inches in diameter. Each was formed from a celluloid disk by cutting out seven narrow strips $\frac{1}{4}$ inch in width to within $\frac{1}{4}$ inch of the circumference.
 - (d) 30 pieces of unbleached sponge to place in the small end of each cone.
2. A large quantity of circular smooth egg-cards $3\frac{1}{2}$ inches in diameter.
 3. A large quantity of blotting paper in strips ($\frac{5}{8}$ inch \times 8 inches).

In assembling an egg-deposition cage a grating was fastened to a circular cardboard with metal clips so that there was no appreciable space between the two disks. This egg-card was then inserted in a tongue slit cut in the celluloid cone about $2\frac{1}{2}$ inches from its base so that the egg-card was held in a vertical position at right angles to the base. The cone was then placed in its jar, point first, touching the glass only with its base, the cone being held in position by its base being wedged at the opening of the jar. When the cones had been in use several days the blotting-paper strips were employed to help bind the cones in place. A single strip was wedged in between the cone and the jar.

The moths on the room surfaces and on the under-surfaces of the bin covers were gathered a few at a time in a large vial and quickly dumped into an egg-deposition cage through the interior of the cone until about 400 were trapped in the cage. As each cage was stocked a sponge saturated with water was placed in the small opening of the cone to provide the moths with moisture and to prevent them from gaining access to the interior of the cone, which formed the exterior of the cage. The cage was then inverted and set aside for 24 hours.

When thirty egg-deposition cages were used they were handled as follows:

Twenty cages were kept stocked continuously. Each morning the moths in these were transferred to the ten reserve cages. After the used cages were taken apart, cleaned, and reassembled, ten of them were restocked with new moths, leaving ten cages in reserve for the following morning.

The transfer of the moths was accomplished by removing the cone and strip, placing a sheet of paper over the jar to retain the moths, inverting the jar over a freshly made up cage, removing the paper and blowing forcibly between the narrow space between the jars.

The moths deposited an average of 1,000 eggs on the egg-card beneath the celluloid grate and quantities on the strip at the base. All loose eggs deposited in the moth debris were collected, sifted, and scattered evenly over egg-cards covered with shellac.

This equipment was too cumbersome and it was decided to decrease the equipment and at the same time increase production.

A suction cap connected with a vacuum pump was designed which fitted over the jars and enabled the operator to collect moths with a great saving of time. Glass shelves were substituted for the cardboard bases of the parasite cages and eliminated a source of trouble.

The fact that the moths also oviposited in debris at the bottom of the cages led to the development of a greatly simplified method of obtaining eggs. In May, 1927, the battery jars were replaced by two cardboard cylinders capped on each end with 20-mesh brass sieves. Sufficient moths were collected and dumped in these cages to cover the bottom. The crevices formed by their bodies stimulated egg deposition. By this means free eggs were obtained. This was desirable for several reasons. The free eggs could be evenly distributed in a single layer over cards of uniform size and thus afford a fairly accurate means of measuring production. When thus fastened to the egg-card the danger of a larva hatching from an unparasitized egg and accidentally destroying parasitized eggs around it was negligible. A moth ovipositing in a crevice deposits her eggs in a horizontal position in a compact mass. The larva emerging at the cephalic pole of the egg may pass through a number of parasitized eggs.

Collection of the moths was facilitated by placing light boards across the top of each bin so that they were in contact with the corn. Each board measured 3 feet \times 5 inches \times $\frac{1}{4}$ inch. The moths gathered in numbers on the ventral surface of the board. When collecting the moths each board was removed and the moths scooped up with the collecting hose.

With these changes in method parasite production increased to 200,000 daily in July, 1927. In order to increase the capacity of the insectary for 1928 production, a more compact type of corn bin was designed which permitted the use of at least three times the amount of corn possible with the open bins in the same amount of space.

A description of the equipment used in 1928 follows:

The insectary of the Saticoy Walnut Growers' Association, where *Trichogramma* production was initiated (fig. 2), was divided into two compartments: the laboratory-office and the rearing room. The rearing-room ceiling and walls were lined with plaster board and the floor was of wood. The inside dimensions were: length 17 feet, width 15½ feet, height 8 feet. Two windows, 24 inches high, extended the length of each side. Circulation of air was obtained by means of a rotary ventilator which operates whenever the wind velocity exceeds

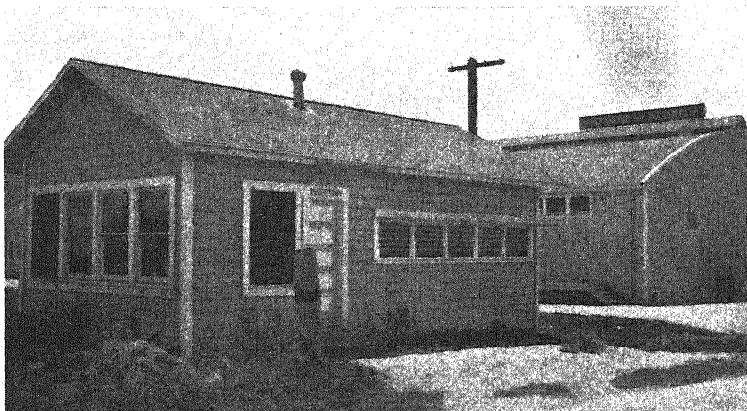


Fig. 2. Insectary of the Saticoy Walnut Growers' Association, where mass production of *Trichogramma* originated.

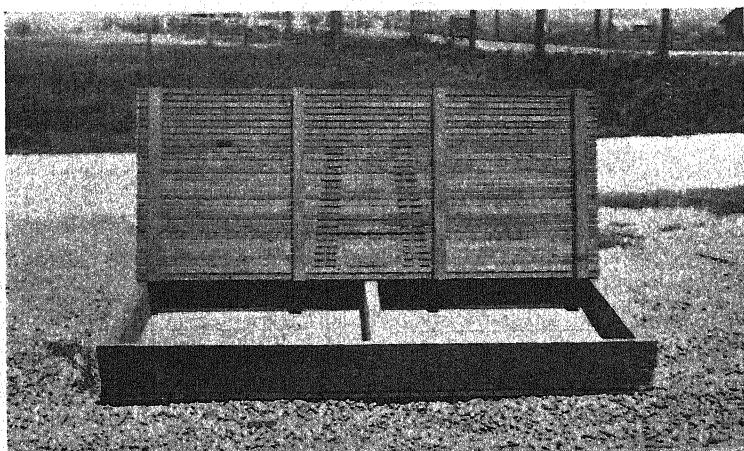


Fig. 3. Type of bin unit adopted in 1928.

two miles an hour. The air passed down through floor openings and upward through an 8-inch pipe that extended above the roof. The room was heated by eight electric heating units controlled by a thermostat.

The grain containers (fig. 3) were shallow bins with covers made of strong slats set $\frac{1}{8}$ inch apart. Each bin was 5 feet long, 2 feet wide, and 4 inches deep, with all outside surfaces smooth. The slats were $\frac{3}{4}$ inch wide and $\frac{3}{4}$ inch thick and surfaced on three sides. The rough

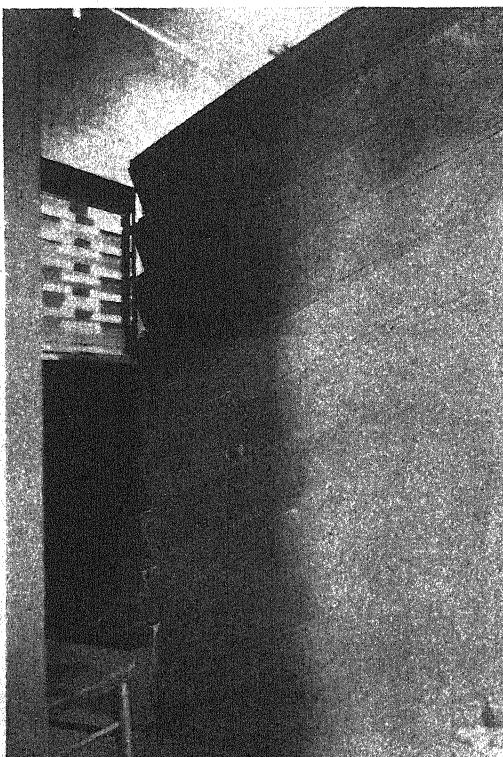


Fig. 4. Side view of bins in position.

side formed the inner surface of the cover. To save construction costs the bins were not constructed to permit filling and emptying without removing from the stacks.

As the bins were placed in the rearing room they were filled with corn and the covers fitted into place and fastened by dowels. The bins were stacked 10 high crosswise of the room (fig. 4), forming two rows lengthwise of 60 bins each. The inner end of each row rested on a baseboard 22 inches high and the outer end on the floor, so that

the bins were set at an angle with the floor of about 25 degrees. If this angle is 22 degrees or less bin covers are not needed to hold the corn in place. The bin stacks and enclosures occupied a floor space 13 feet by 12 feet. A solid wooden cover was placed on the top bin of each stack.

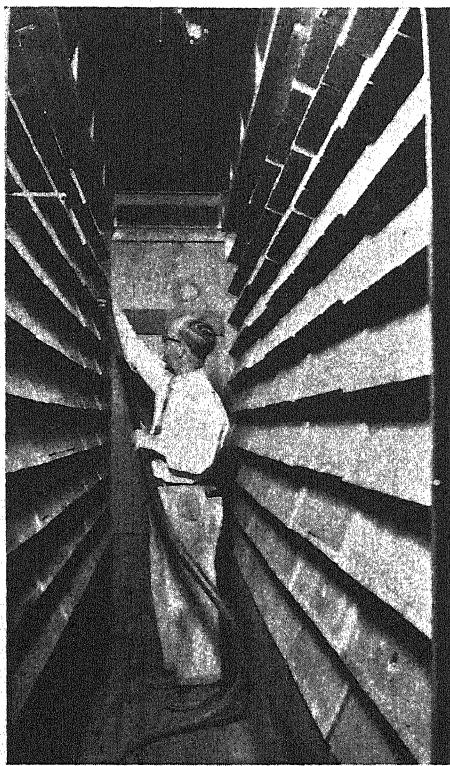


Fig. 5. Moth enclosure between stacks of bins.

The moth enclosure (fig. 5) was 13 feet long, 26 inches wide, and 8 feet high. The air entered the enclosure through ventilators and passed downward between the bins into the surrounding room. All the moths emerging from the grain accumulated in the moth enclosure. Crevices other than those leading into the grain were filled with putty.

The moths were drawn into a 1½-inch hose 17 feet long through a soft-nosed nozzle inserted in the end of the hose. The nozzle used was a standard vacuum-cleaner attachment, one end of which is compressed to make an opening 2 inches long and ¼ inch wide. The hose led

into a receptacle (fig. 6) consisting of a gallon battery jar containing an interiorly directed truncated celluloid cone and a tin cover 6 inches in diameter and 4 inches high. The cover was provided with an inlet terminating a short distance above the smaller end of the cone and below a horizontal wire screen above which was an outlet that connected with the source of partial vacuum. The moths were prevented from passing out of the receptacle through the outlet by the wire



Fig. 6. Entrance to moth enclosure and moth trap in position.

screen and were retained in the jar by the cone. The wire screen also served to hold the inlet tube in position. The jar could be removed from under the metal cover without loss of moths.

After removal the jar was shaken sharply to cause the moths to settle in the bottom. The moths were then dumped into the egg-deposition cages (fig. 7). A strong current of air passing across the top of the cage tends to inhibit the flight of the moths. The 20-mesh screen will allow many of the males to escape.

The egg-deposition cages consisted of smooth cardboard cylinders capped on each end with 20-mesh brass sieves. Enough moths were placed in each cylinder to cover the bottom screen so as to prevent any light from filtering through. The cages were then set over a truncated trough (fig. 8) beneath which was placed a sheet of paper to catch the eggs dropping through. A mild current of air was circulated beneath the cages to carry away the scale dust.

The equipment for preparing the eggs for parasitism (fig. 10) consisted of 18- and 30-mesh wire strainers, moth-rinsing jar, filter

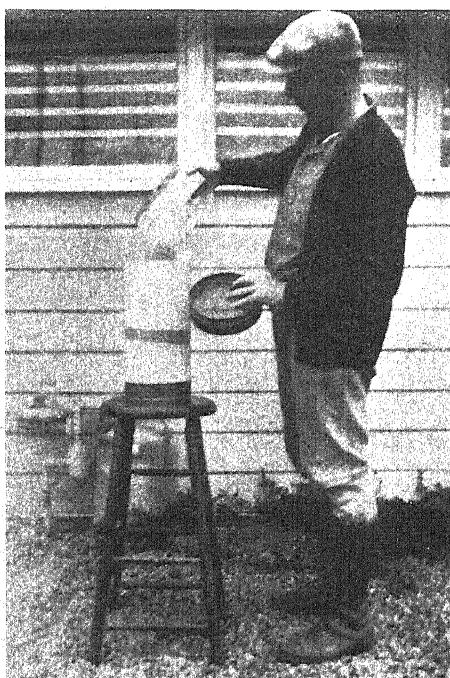


Fig. 7. Emptying moths from trap into egg-deposition cage.

paper and funnel, shellac, brushes, and cardboard disks or egg-cards. The diameter of each egg-card was $3\frac{1}{2}$ inches; that of the aperture in the center $\frac{3}{4}$ inch. When the eggs were evenly distributed over the card the approximate number of eggs could be determined by multiplying the number of eggs visible in the low-power field of the binocular microscope by the number of times the area of the card exceeded that of the microscope field. The cards were placed on spindles (fig. 11) for storage and shipment in mailing tubes.

The inner face of each window in the rearing room was lined with a tier of six plate-glass shelves 5 inches wide (fig. 12). The shelf capacity was sufficient for 600 petri-dish cages. These were selected for their even edges so that when inverted on plate glass they would make contact at every point and prevent the escape of the parasites. The inside diameter of the petri-dish cages was $3\frac{3}{4}$ inches and they



Fig. 8. Egg-deposition cages in position over trough.

easily covered the $3\frac{1}{2}$ -inch egg-cards. With this type of cage the egg-cards can be quickly removed and replaced with practically no loss of parasites.

An electric refrigerator was used for the cold storage of the parasitized eggs. The freezing unit was "de-iced" daily in order that the refrigerator might be used continuously. The dehydrating effect of the freezing unit in operation can be minimized by placing the parasitized eggs in small air-tight containers in the storage compartment.



Fig. 9. Separating eggs from moth debris before winnowing.
(Photo from Los Angeles Times.)

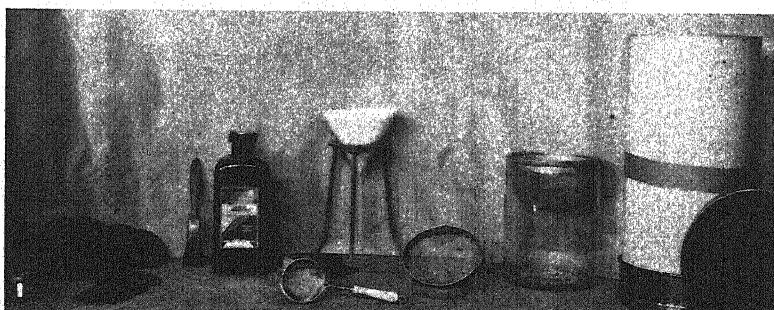


Fig. 10. Equipment for separating eggs and preparing egg-cards.

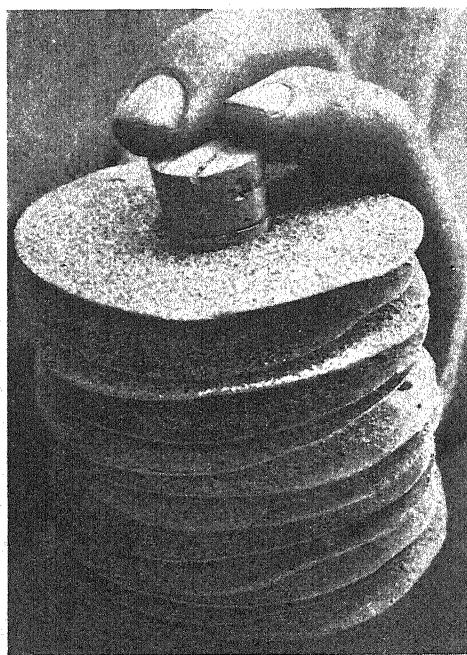


Fig. 11. Egg-cards on spindle for convenience in handling.
(Photo from Los Angeles Times.)

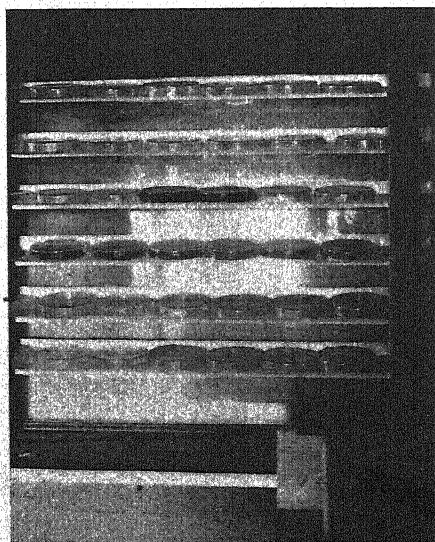


Fig. 12. Parasite cages in position on plate-glass shelves.

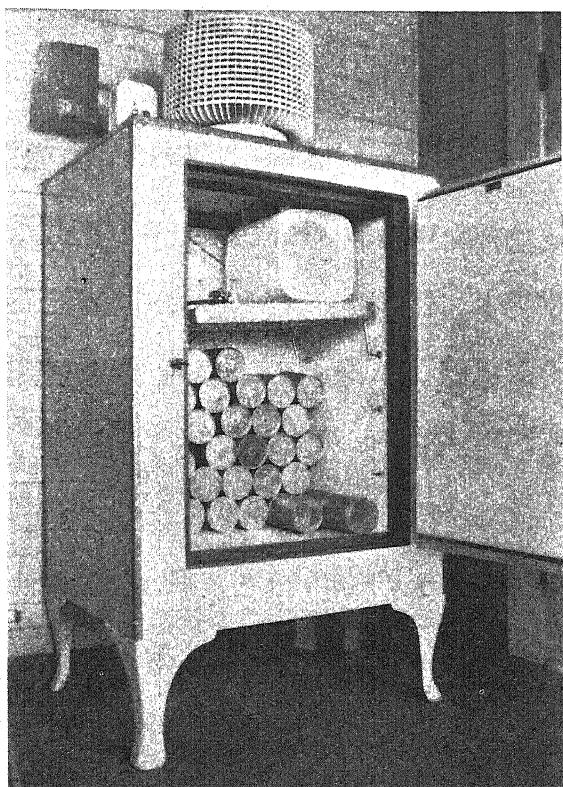


Fig. 13. Mailing tubes containing parasitized eggs in cold storage.

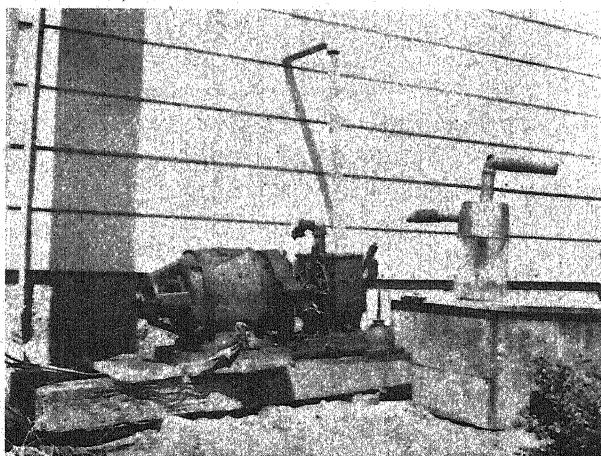


Fig 14. Lammert compressor as a source of suction for moth collection.
On the box is the type of trap used.

The Lammert compressor (fig. 14) was connected with the moth trap by 17 feet of 1-inch pipe. It was operated by a 1½ H.P. motor, and the full amount of suction when used with a 1¼-inch collecting hose did not injure the moths.

A Royal dryer can be used instead of the compressor. It has the advantage of being portable. It can be fastened to the suction cap as in figure 17.

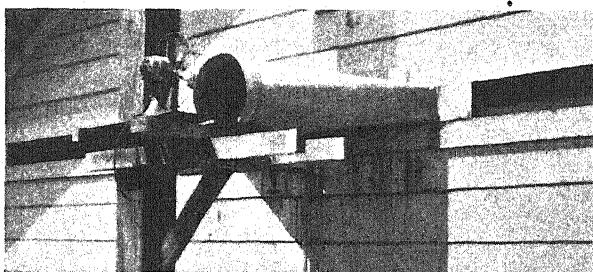


Fig. 15. Humidifier on outside of insectary.

The humidifier (fig. 15) was set in operation when the humidity in the moth enclosure dropped below 55 per cent. This apparatus consisted of a flattened sheet-iron funnel about 30 inches in length. A very small stream of water under high pressure was injected through the bottom of the funnel. This was broken into a spray and vaporized by passing it through an 80-mesh screen. A fan forced the moisture laden air into the moth enclosure through the ventilator. Two wire screen 4 inches apart in the large end of the funnel served to collect the excess water in the air and pass it outward. The inner screen, which is next to the spray inlet, was made of ordinary fly screening, and the outer one of 20-mesh screen. It is preferable to place the humidifier in the rearing room and recirculate the room air. A more uniform vaporization is obtained.

REARING EXPERIENCES IN 1928

In preparation for the 1928 production ½ ton of white corn was vacuum-fumigated with carbon disulfide for 24 hours and then placed in one of the rooms of the county insectary August 10, 1927. The temperature of this room was maintained at 80° F. Beginning several days later thousands of *Sitotroga* eggs were placed on the corn daily for a period of 20 days. Moths appeared at the end of 24 days.

By the first of October the old infested corn had been removed from the rearing room and sold for stock feed. Pending the resumption of moth production a million parasitized eggs were placed in cold storage in the local meat market.

Upon completion of the new bins the first of November the half ton of newly infested corn and an additional ton of uninfested corn were placed in the rearing room. Unfortunately, the precautions taken to prevent an infestation of the black weevil, *Sitophilus oryzae* L., had not been sufficient, for several specimens were found in the bottom of the containers. These became so numerous by March as to be troublesome during the collection of the moths.

On December 7 the remaining bins were filled with $7\frac{1}{2}$ tons of newly harvested corn. Some of the corn, which at the time felt heavy with moisture when held in the hand, later developed mold to the extent that infestation by the moths was prevented in eighteen of the bins. Moths began to emerge from the rest of the corn the first week in January. One hundred kernels from the upper part of one bin showed an infestation of 30 per cent on January 30.

At this time parasite production recommenced. By the first week in February 150,000 eggs were collected daily and parasitized. The number of moths then began gradually to decrease.

In order to bring about an increase, all of the eggs collected during the first 5 days in March were blown back into the bins. As a result there was a marked increase on March 28, and during the first week in April an egg production of 400,000 was attained. This peak, however, was followed by a rapid drop in production until by the middle of April the daily production was less than 50,000. During a 3-day period ending April 15 all of the eggs were again replaced.

On April 20 it was found that the corn contained only about 6 per cent of moisture. The rapid circulation of air maintained to prevent the corn from molding had evidently been continued too long. An indicator that should have received recognition was the foreign grain beetle, *Cathartus advena* Waltl. This fungus feeder was very abundant while the mold was developing on the corn but about the middle of March it suddenly disappeared.

In order to rapidly raise the moisture content of the corn the entire 9 tons was removed, run through a fanning mill, sprinkled with quantities of water, and then replaced. This procedure occupied 5 days beginning April 24 and raised the moisture content of the corn about 3 per cent.

The percentage of infestation in the corn was ascertained at this time. A count of the emergence holes in the kernels showed that the

upper or inner portions of the bin averaged 27 per cent, the middle 6 per cent, and the lower or outer portion 11 per cent. Probably most of this infestation took place during the months of December, January, and February.

On the thirteenth day after the replacement of the corn an egg production of 150,000 was again reached. This occurred 28 days after the second replacement of eggs. After this peak, however, production rapidly declined to zero.

Many moths emerged but hardly had their wings expanded when they dropped to the floor and succumbed to some obscure ailment. The floor was swept twice daily, yet 6 hours after each sweeping it would be covered with moths showing no sign of injury or wear. Many of the moths died in copula and the females were turgid with eggs.

A microscopic examination of the sweepings revealed the presence of the mite, *Pediculoides ventricosus*, in great numbers. It was found in greater abundance on the top surfaces of the bin stacks. It attacked both the egg and the adult. The mite *Tyroglyphus* also disappeared with the advent of *P. ventricosus*.

The last full egg-card to be prepared was found to be infested with many mites. An examination of egg-cards prepared only a week earlier, however, showed no infestation.

This rapid rise in abundance of mites indicates that conditions prior to the removal and replacement of the corn had not been optimum for their development. The high humidity maintained after the installation of the humidifier on April 28 may have been the immediate cause of the outbreak. The relative humidity was maintained at an average of approximately 70 per cent, 30 per cent higher than was maintained before the installation of the humidifier.

The mites, however, had probably been present for a considerable time, for dead moths had been observed on the floor in March. It is possible that mites instead of lack of moisture were responsible for the earlier reduction in moth production.

The mites are very susceptible to sulfur fumes. On May 19 the 9 tons of corn were fumigated with 12 pounds of liquid sulfur dioxide. For several days thereafter it was impossible to find any mites, but a week later they were again plentiful. Sulfur dioxide apparently acts as a repellent and also as a poison to the black weevil. It caused great numbers of them to leave the bins and their audible gnawing of the corn ceased almost entirely. Ten more pounds of sulfur dioxide were applied late in June against the mites, greatly reducing their numbers.

All of the corn was again removed July 17. On this occasion it was noted that all of the black weevils in many of the bins were dead.

From the beginning conditions in the rearing room were more favorable to the development of the *Sitotroga* than for either *Ephestia keuhniella* or *Plodia interpunctella*, for although the latter were present in small numbers early in the season they disappeared almost entirely. Since *Sitotroga* larvae occasionally attack each other, they may have been the cause of the disappearance of the other moths.

The destructive weevil can be eliminated by the sterilization of the rearing room and vacuum fumigation of the new stock of grain prior to its inoculation with *Sitotroga*.

In September the used corn was sold for \$1.95 a hundred pounds and 5 tons of new corn purchased. This was fumigated in a vacuum for 48 hours with hydrocyanic-acid gas and for 48 hours with carbon bisulfide. The rearing room was cleaned and then fumigated with liquid sulfur dioxide and hydrocyanic-acid gas. On October 7 the upper halves of the bins were filled with a total of 9,300 pounds of corn. During the following month almost a million moth eggs were placed on the corn. The eggs were obtained from a half ton of infested corn at the county insectary. The room was kept heated artificially until the heat of infestation was sufficient to keep the temperature in the bins at 70° to 95° F, which according to Back⁽¹⁾ is high enough for rapid development. With production under way it is desirable that the air into which the moths emerge should be cooler than the corn. The moth-rearing room should, therefore, be separate from the room used for moth egg-deposition and parasitism. As Schulze⁽²⁰⁾ found, a temperature of 81° F is optimum for *Trichogramma* propagation.

The daily collection of moths for egg production commenced December 10. Production increased until on January 20 the collection of eggs reached 600,000 daily. A month later, however, *Pediculoides* stopped egg production entirely. It is possible that insects such as the cadelle beetle and flat grain beetle are carriers of this mite and were responsible for its introduction to the rearing room.

The mite hazard could probably be reduced to a minimum by building up an infestation in eight weeks to a level at which egg production is sufficient for economical parasite propagation, i.e., 100,000 eggs daily per ton of corn used, maintaining this rate of production for about four weeks, and then fumigating the corn and reinfesting it or renewing with fresh corn.

To insure uninterrupted production auxiliary rearing rooms are necessary.

DAILY PROCEDURE IN PRODUCTION IN 1928

The procedure followed in the daily routine of production was as follows:

Once every 24 hours the eggs in the trough beneath the egg cages were collected, screened, and winnowed of moth appendages and scales. The cages were lightly shaken to dislodge eggs adhering to the moths.

The accumulated eggs were then poured onto freshly shellacked cardboard disks and all of the eggs not adhering were shaken off. The shellac at the moment of applying the eggs must be sticky enough to hold the eggs but not so fluid as to engulf them. It is best applied with a small brush.

After allowing the cards to dry for about half an hour or until the alcohol in the shellac has evaporated, they were placed in the parasite cages for a period of 24 hours with night illumination.

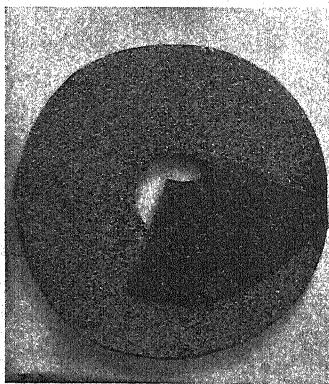


Fig. 16. A fresh egg-card and segment of emergence card.
(Photo from Los Angeles Times.)

Before the introduction of the new egg-cards, one-third of a card (fig. 16), from which parasites had begun to emerge, was placed in the parasite cages to provide the stock required to impregnate at least 90 per cent of the new eggs.

Each emergence card was used for two days so that a six-fold increase was obtained.

At the end of 24 hours the new egg-cards were removed and suspended on hooks to aid the negatively phototropic and positively geotropic larvae from unparasitized eggs to leave the cards. Three

days later the parasitized eggs had turned black, an indication that the parasites were in the prepupal stage. The cards were then placed in cold storage (fig. 13), one-sixth being retained for emergence cards.

After the collection of eggs and the preparation of the parasite cages, the day's crop of moths was gathered by means of the vacuum collector and dumped into the egg-deposition chamber.

After remaining in the egg-deposition chamber 2 days the moths were poured into a wire strainer and rinsed under water pressure to remove the adhering eggs. These eggs settled to the bottom of the container and the debris rising to the surface was drained off. The eggs were then separated out by means of filter paper or an 80-mesh brass sieve from which, after drying, the eggs were easily brushed off. Thousands of eggs were conserved in this manner in addition to those collected daily from beneath each cage.

When mailing cans were used for parasite cages the egg-cards were made in oblong sheets that fitted closely to the inside surface of the cages. These cards were pinned in position. Each card carried on one side about 150,000 eggs, or 6 eggs to each square millimeter. This type of cage was used in the same manner as the petri-dish cage. When inverted on the plate-glass shelves the metal bottom of the container reflected the light from below. Mercury vapor tubes 24 inches long fastened to the ventral surface of the glass shelves may be used for illumination.

TRICHOGRAMMA PRODUCTION IN MEXICO

In the spring of 1929 the writer, at the request of Señor Diego Redo, initiated the production of *Trichogramma* on his sugar plantation on the west coast of Mexico. The native strain of *Trichogramma* was used. The initial stock was obtained the last of February from parasitized eggs of the milkweed butterfly, *Danaus menippe* (Hbn.). A great quantity of corn, shelled and on the ear, stored in bulk in the plantation warehouse, was found to be heavily infested with many kinds of grain pests, but chiefly *Sitotroga*. The burlap method was devised for collecting the moths. Burlap sacks were laid out evenly on the surface of the corn. Moths congregated on the ventral surface in numbers. Once or twice a day each sack was lifted clear of the grain and looped to form a funnel, the lower end encircling a cylindrical receptacle made of 20-mesh brass screen about 8 inches in diameter. Several sharp blows on the burlap precipitated the moths into the screen container. This container was then used as an egg-

deposition cage. It was placed in a housing so constructed that when a strong current of air was directed on it, oviposition was stimulated. Eggs not adhering to the outside surface of the screen dropped into a trough beneath. The outside of the cylinder was often white with eggs which were easily brushed off.

By the first of June the laboratory operator reported a daily production of 400,000.

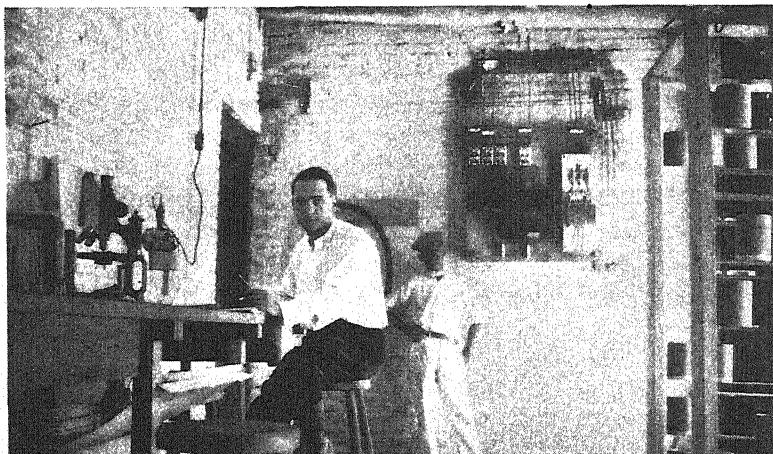


Fig. 17. Interior of the Redo y Cia Insectary at Eldorado, Mexico. Note the plate-glass shelves and the mailing tubes used as parasite cages.

The equipment used in handling the host eggs and the parasites was as follows:

FOR HANDLING EGGS

- 2 20-mesh screen cylinders (8×18 inches) with removable cap.
- 1 electric fan.
- 1 26-mesh wire strainer.
- 1 18-mesh wire strainer.
- 1 roll of mechanics pattern paper for egg-cards.
- 1 pair of shears.
- 1 gallon of white shellac.
- 1 brush for applying shellac.
- 1 brush for removing eggs from egg-deposition cylinders.
- 1 16-mesh strainer (6 inches in diameter in which to wash dead moths).
- 1 80-mesh brass sieve to strain out eggs washed from moths.
- 1 brush to remove eggs when dried.
- 2 glass gallon jars (6 inches in diameter).

FOR COLLECTING MOTHS

A number of burlap sacks.

- 1 Royal electric dryer mounted on a suction cap fitted to a glass gallon jar and having a $1\frac{1}{4}$ -inch hose 5 feet in length attached.

FOR PARASITIZING EQUIPMENT

50 feet of plate glass 4 to 5 inches in width.

2 dozen selected glass petri dishes (4 inches in diameter).

12 dozen mailing tubes ($5 \times 3\frac{1}{2}$ inches) with metal bottoms.

Several sheets of emery paper to smooth down rims of the mailing tubes used as parasite cages.

6 dozen rolls of fine wire.

STORAGE OF *TRICHOGRAMMA*

The optimum conditions for the cold storage of *Trichogramma minutum* have yet to be worked out. Fiske⁽¹⁰⁾ kept parasitized eggs successfully for about ten months at temperatures of 28° to 30° F, placing them in cold storage the moment they began to turn black. He found⁽¹²⁾ that if the young parasites are subjected to a low temperature their development is delayed many days even though exposed later to continuous high temperatures.

Mokrzecki and Bragina⁽¹⁶⁾ were able to maintain *Trichogramma semblidis* in cold storage for ten months. They reported that hibernation sets in at 38° to 39° F. Zorin⁽²⁶⁾ found that the larvae of *T. evanescens* when kept at a temperature of 50° and 51.8° F enter the diapause and do not pupate.

Trichogramma reached maturity in 32 days when the average mean daily temperature for the period of development was 55.4° F. Twenty-six days were required at 58° F. The life cycle as determined by Mokrzecki was 38 to 43 days at temperatures from 48° to 52° F.

In March, 1928, a well-parasitized egg-card was sent to E. J. Newcomer at Yakima, Washington. He was notified that the parasites should emerge beginning March 19. This they did, but as he had no host eggs he placed the card in the cellar where the temperature was from 45° to 55° F. A month later he was considerably surprised to find many of the parasites alive.

The cold-storage facilities on trans-Pacific steamers made it possible to successfully transmit several thousand parasitized eggs to Australia in August, 1927.

In two local strains of *Trichogramma* there is a noticeable difference in coloration between the females developing at high temperatures and those at low temperatures. The bodies of the former are light yellow, while the latter are as dark as the males. The strain from a cool moist coastal region turn dark at a higher temperature than the strain from a hot, dry interior valley.

PROCEDURE IN FIELD LIBERATIONS

The procedure in liberation of the *Trichogramma* in the field was as follows:

The egg-cards were placed in the field just as emergence began. The cards were cut into sections of 10,000 eggs and a fine wire several inches in length was attached to each section.

Each section was suspended from the lower portion of the food plant of the host. The parasites are usually negatively geotropic. An egg-card suspended by the fine wire is protected to a greater degree from attack by predators than when in contact with the plant.

In timing the sequence of field liberations an increase or decrease of one degree in the mean daily temperature was considered as shortening or lengthening the developmental period one day.

REACTION OF TRICHOGRAMMA TO FIELD CONDITIONS

Complete emergence in the field required usually from 3 to 6 days. After 6 days the cards were examined to determine the amount of emergence. The fact that the males remain on the card, attracted by the emerging females, insures reproduction in the field, for unmated females produce only males. Male parasites may be found on the cards long after the females have left.

The rate of dispersion from the point of liberation varied directly with the daytime temperature and light intensity and inversely with the excessive air movement and surface moisture.

For a period of 8 days many parasites were found on the ventral surface of leaves in the immediate vicinity of an egg-card when the mean daily temperature had been 58° F. If the weather was cool and cloudy the parasites crawled up the wire in search of protected ventral surfaces.

In the field optimum temperature conditions for the increase of *Trichogramma* may be at a lower level than that which is optimum

for the development of its host. For any increase to take place, however, it is essential that the distribution of the host eggs be dense enough to permit the parasite population bridging the gaps between them. The rate of increase tends to vary inversely as the square of the average distance separating the hosts.

At Saticoy where the normal mean temperature in summer is about 65° F a high degree of codling-moth parasitism is obtained by the liberation of *Trichogramma*. The comparatively low temperature and corresponding high humidity prolongs the life of the parasites, increasing the amount of ovulation and the chance of finding a larger number of host eggs than may occur when temperatures are higher and host eggs more abundant. Schulze⁽²⁰⁾ found that the life of the adult seldom exceeded one day at 89.6° F but at 77° F they lived an average of 13 days. Hase⁽⁹⁾ kept adults alive with an abundance of food and at room temperature for 30 days. At high temperature and low humidity the activity of *Trichogramma* appears excessive and aimless. Mokrzecki and Bragina⁽¹⁶⁾ found that in the laboratory temperature of 95° to 100° F were fatal.

According to Barber⁽²⁾ *Trichogramma* appears to parasitize the European corn borer most effectively in cool seasons. He states that "the probable longer periods of life of adults [of the corn borer], caused by low temperatures, served to spread oviposition over a relatively longer period, which was favorable for the development of the egg parasite *Trichogramma minutum* Riley, and the relatively fewer eggs deposited per female probably served to increase the importance of the parasite." That is, in the cooler seasons the more uniform supply of host eggs insures the increase of *Trichogramma* and the smaller number of eggs results in a higher percentage of parasitism. The most important effect, however, is the lengthening of the incubation period of the host egg, exposing it to attack for a longer period. The temperatures at which parasitism takes place range from about 60° to 100° F. The developmental range of the parasite is from about 40° to 100° F. It is therefore probable that in some of its host relations the sphere of activity of each parasite and the percentage of parasitism is greatest when the daily temperatures are somewhat below the optimum for the increase of its host. The actual amount of parasitism, however, is determined by the host population.

EFFECT OF LIBERATIONS

In determining the effect of liberations the natural parasitism must be taken into consideration.

The earliest record of codling-moth parasitism in the vicinity of Saticoy was made in 1924. On a walnut tree in the center of a 70-acre grove 348 eggs were found to be 5 per cent parasitized. Most of this parasitism was due to *Prospaltella* sp.⁵ In 1926 on a walnut tree 461 eggs were found to be 49 per cent parasitized.

TABLE 1
NATURAL AND ARTIFICIAL PARASITISM DURING A THREE-YEAR PERIOD ON ONE TREE
NEAR THE CENTER OF A 30-ACRE WALNUT GROVE

		Weekly inspection								
		June		July				August		
1926 Natural parasitism	Live eggs.....	68	61	154	196	131	48	35	86	1
	Hatched eggs (no record).....									
	Black parasitized.....	0	5	20	29	38	15	12	5	0
1927 Artificial parasitism	Live eggs.....				50	54	11	3	7	0
	Hatched eggs.....				0	5	34	14	0	0
	Black parasitized.....				0	26	51	20	17	4
1928 Artificial parasitism	Live eggs.....		64	279	156	131	171		107	
	Hatched eggs.....		20	35	19	119	116		105	
	Black parasitized.....		2	3	53	184	52		23	

In 1927 40,000 *Trichogramma* were liberated one week prior to the first inspection.

In 1928 9,000 *Trichogramma* were liberated at the first inspection.

Black parasitized eggs appear the second week after liberation.

Natural parasitism on the walnut codling moth in 1927 and 1928 was very light.

In 1927⁽⁵⁾ and 1928 the natural parasitism of codling-moth eggs on walnut trees was very light. Hundreds of walnut trees were closely examined each year by four trained inspectors. The highest number of parasitized eggs found on any walnut tree since 1926 was 12 and these were all due to *Prospaltella*.

In 1928 it was found that the parasitism of codling-moth eggs on dooryard pears and apples was in some cases nearly 50 per cent.

⁵ All the known species in this genus of parasites attack armored scales or white flies. That its attack on the codling-moth egg is accidental is borne out by the fact that so far only males have been obtained. It is a factor, however, in the evaluation of natural parasitism. Unlike the sac-like larva of *Trichogramma*, the larva of *Prospaltella* is eel-like and more active and voids solid meconium prior to pupation. The presence of this excrement indicates *Prospaltella* as having parasitized the egg.

Several hundred of the parasitized eggs were examined and it was found that 30 per cent of the parasitism was due to *Prospaltella*.

Observations were made of the parasitism on one walnut tree for three successive years (table 1). Each year codling-moth eggs were found on this tree in greater numbers than on any other tree in the vicinity. Parasitism on surrounding trees was very light.

In 1927 many thousands of *Trichogramma* were liberated in the walnut groves of the Association but accurate checks upon the percentage of parasitism were made only on six trees (table 2). The percentage of parasitism was first ascertained about two weeks after the first liberation when the parasitized eggs turn black. The effect of the release of *Trichogramma* was to increase the percentage of parasitism in three weeks from less than 1 per cent up to as high as 52.4 per cent on the most highly infested trees.

TABLE 2

PARASITISM RESULTING FROM THE LIBERATION OF APPROXIMATELY 100,000
Trichogramma ON SIX WALNUT TREES JUNE 27 AND JULY 6, 1927

Tree	A	B	C	D	E	F	Average
Number liberated.....	25,000	15,000	10,000	15,000	10,000	25,000	16,666.6
Hatched eggs.....	127	68	124	94	88	109	101.6
Parasitized.....	140	26	30	66	41	47	58.3
Per cent parasitized.....	52.4	27.6	19.5	41.2	31.8	30.1	36.4

Natural parasitism was less than 1 per cent.

The cards were placed on the trees 1 day prior to emergence. The amount of emergence was not determined.

In 1928 fewer parasites were available owing to the destruction of the laboratory host by *Pediculoides*. Cold-storage parasites, however, were liberated on one highly infested walnut tree and also on nine apples and pears on the Lloyd-Butler Ranch. As in 1927 a marked increase in parasitism followed the liberation (table 3). A comparison was made between the artificial parasitism obtained on the pears and apples on the Lloyd-Butler Ranch and the natural parasitism on check pear and apple trees located 3 miles distant. The parasitism on these check trees was the highest of any trees examined for use as checks. The highest natural parasitism was 45.7 per cent and the highest artificial parasitism was 72.9 per cent. The parasitism on each tree in the test plot was higher than 45.7 per cent, the maximum natural parasitism on the check trees. Because of the natural parasitism the influence of the liberated parasites on the total parasitism was probably greatly reduced.

In May, 1928, the writer sent 40,000 parasitized eggs to C. H. Alden in Georgia. In June he reported that the parasites emerging from them were very effective; over 200 codling-moth eggs were parasitized in approximately 220 eggs under observation.

TABLE 3

NATURAL PLUS ARTIFICIAL PARASITISM IN 1928 ON DOOR-YARD APPLE AND PEAR TREES DURING FIRST-BROOD EGG DEPOSITION

Summary of observations made each week from May 25 to June 29 on pear

	P1*	P2	P3	P4	P5	P6	P7	C1†	C2	C3
Number liberated.....	1,200	3,800	4,500	1,200	2,500
Date of liberation.....	May 5	May 5	May 13	May 5	May 5
Hatched.....	63	145	154	105	49	45	22	157	110	139
Parasitized.....	170	161	257	267	67	72	19	75	60	117
Per cent.....	72.9	52.6	62.5	71.7	57.5	61.5	46.3	32.3	35.3	45.7

Summary of observations made each week from June 1 to June 29 on apple

	A1*	A2	A3	A4	†C4
Number liberated.....	3,000	3,800	2,500	3,500
Date of liberation.....	May 13	May 5-13	May 13-20	May 5-15
Hatched.....	56	47	92	60	103
Parasitized.....	121	80	156	83	18
Per cent.....	68.3	63.0	62.9	58.0	14.8

* All trees marked *P* and *A* form a small door-yard orchard on the Lloyd-Butler Ranch.

† All trees marked *C* are located three miles west of trees *A* and *P*.

Cards were placed on the trees the day emergence began.

POSSIBILITIES IN THE USE OF *TRICHOGRAMMA*

The possibilities in the practical use of *Trichogramma* in biological control work deserves full investigation, since the production of this parasite in large quantities is now feasible.

Within the last two years over a score of entomologists have turned their attention to *Trichogramma*, and are investigating its use against the codling moth, the European corn borer, sugar-cane borer, the pecan nut case-borer, the corn ear worm, celery leaf-tyer, tea tortrix, oriental peach moth, rice moth, and cabbage butterfly.

In case of field-crop insects such as the European corn borer, early mass liberations when host eggs are fairly abundant and there is no natural parasitism may result in the building up of a parasite population early in the season. In such a case success is dependent on the natural accretion.

To employ this *accretive method* in the field when host eggs are comparatively scarce is useless. If liberations are delayed until the host eggs become abundant the acceleration of the natural parasitism probably would not be affected by the addition of more parasites. The saturation point of *Trichogramma* is not likely to be advanced since its survival potential decreases as its density of population increases above a certain point. The saturation point appears to be determined by unknown environmental complexes. For the parasite to be of value its population at this saturation point must be dense enough to prevent the host from becoming economically injurious. The most likely means by which the degree of parasitism may be built up earlier in the season is through utilizing egg-concentration centers. In the case of night-flying hosts having females which are in some degree positively phototropic, egg deposition may be concentrated in the vicinity of lighted points in the infested area. *Trichogramma* could then be colonized at such focal points and an early increase in parasitism started.

In the control of orchard insects, such as the codling moth or orange tortrix, parasites should be liberated each week or twice a week during the first part of the egg-deposition period of the moth, including the peak of egg deposition, possibly 10,000 to 50,000 being placed on each tree during the season. This type of control is analogous to spraying and dusting in that a greater amount of lethal material is used than is actually effective, that repetition may be necessary, and that the effect is more or less immediate. This might be called the *inundative method*.

In the control of the codling moth on apple trees the first effort will be to substitute *Trichogramma* for the cover-spray applications of lead arsenate and thus eliminate the arsenical residue problem.

The basis for commercial control by means of this parasite is mass production at a low cost. It is probable that in the near future improved methods in rearing will reduce the cost of *Trichogramma* production to less than \$10.00 per million.

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